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A SYSTEM OF BACTERIOLOGY IN RELATION TO MEDICINE

VOLUME II

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CHAPTER I. THE STAPHYLOCOCCI.

By Alexander Fleming
(St. Mary's Hospital, London).

With a Section by W. Bulloch.

The staphylococci are a group of Gram-positive microbes characterized by and christened because of their arrangement in irregular masses which have been likened to a bunch of grapes.

History.

By W. Bulloch, London Hospital.

The occurrence of cocci (κόκκος—a seed) in inflammatory processes was observed by many workers during the seventies of last century. Following Billroth (1874) these cocci were differentiated by their size or arrangement (micrococcus, streptococcus, diplococcus). They, along with other forms, were believed by Billroth to belong to a single organism—*Coccobacteria septica*. In his classical work on the ætiology of traumatic infective diseases, R. Koch (1878) showed that this view was untenable. He described six different infective diseases induced in animals experimentally. He wished to extend his studies to traumatic infection in man but was not placed in a position to do this.

The hiatus was filled by A. Ogston (1880, 1881, 1883), a surgeon of Aberdeen, who, utilizing the same methods as Koch, examined the pus of no fewer than 100 abscesses. He found cocci in all of them except 13, which were of the chronic or 'cold' type. The cocci were present alone in 88 per cent. of the cases, while in the remaining 12 per cent. they were associated with bacilli, bacteria or spirilla. Ogston endeavoured to show —what at that time was not proved—that these cocci were the cause of the suppuration. He found that inoculation of pus containing cocci was followed in animals by the development of abscesses, whereas pus from 'cold' abscesses which apparently contained no cocci had not this effect. Pus containing cocci was rendered inert by heat or by antiseptics. Ogston also grew the cocci from abscesses in eggs and showed that they greatly increased in numbers. He was able to produce pyogenic lesions with the cultures diluted 146 millionfold. He distinguished two kinds of cocci, viz. those in groups and in chains, and he correctly inferred that the difference in arrangement was due to a fundamental difference in their respective modes of division. For the group cocci Ogston (1883) introduced the name *Staphylococcus* (σταφυλῆ bunch of grapes) while he retained Billroth's name *Streptococcus* (στρεπτός chain) for the cocci in chains. He regarded the two types as distinct.

The value of Ogston's work was widely recognized, and with the introduction of Koch's pure culture methods was extended by Julius Rosenbach (1884), F. Krause (1884) and Passet (1885). From abscesses and other inflammatory processes Rosenbach cultivated two types of staphylococcus, which he named respectively *Staphylococcus pyogenes aureus* and *albus*. Passet isolated a third type—*S. pyogenes citreus*.

Even before Rosenbach (1884), claims were made by Struck (1883) on behalf of Becker who isolated yellow growths of staphylococcus from osteomyelitis. At first Becker's coccus was regarded as peculiar to osteomyelitis, but between 1884 and 1894 a very large number of observations showed that the yellow growing staphylococcus was the common organism in many other inflammatory lesions and it was shown to play a highly important part in human pathology.

Of great interest and importance was the experimental production in man of pyogenic lesions from the inoculation of staphylococci. The experiments carried out by Garré (1885), Bumm (1885) and Bockhart (1887) on themselves and in other persons settled the pathogenic role of staphylococcus once and for all. W. B.

Morphology.

The staphylococci divide irregularly in two planes, thus arranging themselves in masses, in contrast to the single plane division of the streptococci, which results in chain formation, and to the division in three planes of the sarcinæ, forming cubes and combinations of cubes.

In pus or other infected material they usually occur in irregular groups of deeply staining cocci. There may, however, be many single elements, pairs or tetrads, and sometimes the tetrad formation is very prominent. Frequently the cocci are to be seen inside pus cells and in such cases they may be in all stages of disintegration. The average size is about $0{\cdot}9\mu$, but this varies considerably with the strain and the age of the coccus. In some strains the individuals may not be more than about 0·6 and in others they may average over 1·0. Very young cocci also appear larger than those from a 24-hour culture. The isolated cocci are perfectly spherical, but when occurring in pairs or groups the opposing surfaces may be slightly flattened. Staphylococci are non-motile, have no flagella, capsules or spores.

Arrangement in culture. Different strains vary much in the tenacity with which the cocci hang together ; in many cultures most of the cocci may be single or in pairs, in others tetrad formation may be the most prominent feature, while in others, although there may be some cocci single or in small groups, many larger irregular clumps are present. This last arrangement is seen especially in strains isolated from the skin (Gordon, 1906) and in the ' rough ' variants which can be obtained from many cultures. These organisms do not readily undergo involution, and in 24-hour cultures there is generally a remarkable uniformity in the appearance of the individual cocci.

Staining.

Staphylococci stain well with any of the ordinary bacterial stains. They are strongly Gram-positive. In older cultures some of the cocci lose their power of retaining Gram stain and in pus some of those which have been ingested by the leucocytes may be Gram-negative (an important point to be remembered especially in the diagnosis of gonococcal infections). Growth at high temperatures (43 to 45° C.) renders them partially Gram-negative (Dreyer, 1923).

Cultivation.

The staphylococci are of easy cultivation and grow well on all the ordinary media. They are aerobes and facultative anaerobes. Growth takes place more rapidly and is more copious in the presence of oxygen but it occurs even under strict anaerobic conditions.

The optimum reaction of the medium is pH 6·5, but at a favourable temperature growth takes place between limits of pH 4·5 and 9·8. The optimum temperature is 34 to 36° C., but growth occurs between 10·5 and 48° C. (Walbum, 1922).

In broth, growth usually takes place throughout the medium, rendering the broth evenly turbid. In two or three days, however, a scum appears on the surface. In glucose broth, or broth containing a sugar which is fermented by the cocci there is at first an even growth, but later there is sedimentation of the cocci, leaving the upper portion of the medium comparatively clear. Some strains, especially among those isolated from the human skin or scalp, give a granular growth in broth, which settles to the bottom, leaving the supernatant fluid perfectly clear. Many old broth cultures which show an even turbidity will, when plated thinly on agar, give some more or less irregular colonies which, on being replanted into broth, show this granular growth to a marked degree.

On agar the typical colony is round, regular, slightly raised and very opaque, with a smooth shiny surface. The appearance is rather like a drop of enamel on the surface of the medium. The size of the colonies varies enormously with the conditions, but if widely spaced on a deep layer of medium they may reach a diameter of 4 or 5 mm. With some strains the colonies are very viscid, so that when touched with a platinum wire they can be drawn out into long threads. With others the colony is very adherent to the medium. In old cultures which have not been allowed to dry, daughter colonies may appear. The cultures have a peculiar acrid smell.

Colour.

The colonies on agar may be white, lemon yellow, or any shade of orange up to a deep golden colour. The colour only develops in the presence of oxygen and is most marked when the cocci are grown on an albuminous medium at room temperature for a week or more, exposed to light. Anaerobic growth may induce a permanent loss of chromogenic power (Lubinski, 1894), and the same result may follow heating to 55° C.,

prolonged culture on artificial media, and repeated animal passage (Kolle and Otto, 1902). On an agar plate the presence of a colony of certain saprophytic organisms may markedly increase the coloration of the surrounding staphylococcal colonies.

The staphylococci were originally classified according to the colour of the colony into *aureus*, *citreus* and *albus* types, and there has been much controversy as to whether the types were fixed or whether they could be changed one into the other. This will be further discussed in connection with classification. It is sometimes very difficult to say from simple examination of a culture whether or not it is coloured. Frequently a strain which is known to be of the *aureus* type produces so little colour that it may easily be missed. Probably the most satisfactory method of examination is that used by Winslow and Winslow (1908), who spread a loopful of culture on white paper and noted the colour after it had dried. The yellow pigment belongs to the group of lipochromes. It is insoluble in water but soluble in alcohol, ether, chloroform and acetone. It is changed by strong acids to a blue-green colour.

Growth in Blood and Serum.

On blood agar staphylococcal colonies are usually surrounded by a clear zone of hæmolysis. This is seen whether the culture is derived from an acute lesion or from healthy skin. Grown in human or horse blood in slide cells (vide Vol. IX), they develop in separate woolly colonies around which there is a bluish ring of discoloured blood. In rabbit's blood under the same conditions the colonies are surrounded by a large hæmolytic zone.

Staphylococci planted sparsely in human serum in a capillary tube or in a slide cell do not grow throughout the serum, but remain in woolly colonies. They belong to the group of serophytes (Wright, 1915), i.e. they grow freely in unaltered human serum.

Mutations.

Biggar, Boland and O'Meara (1927), by selection of colonies from old broth cultures of *aureus* types, obtained strains which gave very atypical colonies in one or more of three ways.

(*a*) Colour. *Aureus* strains threw off white colonies which remained white in subculture.

(*b*) Viscidity. Some colonies became very viscid and could be pulled out into long threads.

(*c*) Appearance. The colonies became irregular and ' rough '.

Autolysis.

With some strains if an agar culture is allowed to stand for some weeks the colonies become semi-transparent and in these most of the cocci are degenerate. This may only happen with certain colonies in the culture. Autolysis is more rapid with *S. epidermidis* than with *S. pyogenes* (Hine, 1922). When a 24-hour broth culture of staphylococcus is covered with

a layer of vaseline and incubated, autolysis is rapid, and in 2 or 3 days the turbidity completely disappears. If air is then admitted fresh growth occurs, and if the tube is again incubated anaerobically clearing is only partial (Jaumain, 1922). If a culture is killed by adding to it carbolic acid sufficient to make a concentration of 0·5 per cent. and then incubated anaerobically autolysis is still evident. Autolysis is, however, completely inhibited by the addition of 1 in 6,000 formalin. Heating the culture to 60° C. for 1 hour almost stops the autolysis.

A common laboratory contaminant, a Penicillium, has a very marked lytic action on staphylococci growing in its neighbourhood.

It was with staphylococcus cultures on agar that Twort (1915) first demonstrated the transmissible lytic phenomenon (see Bacteriophage, Vol. VII).

Biochemical Reactions.

Growth in gelatin. In a stab culture a line of growth is seen along the stab in 24 hours, and in 3 or 4 days liquefaction commences at the top of the stab, causing a funnel-shaped depression. The liquefaction spreads downwards until the whole of the gelatin is fluid. Some cultures apparently possess no liquefactive power and generally speaking the *aureus* strains are, in this respect, more active than the *albus* (see Table I).

TABLE I.

Liquefaction of Gelatin by Staphylococci.

Observer	*Percentage of strains liquefying gelatin.*	
	Aureus	*Albus*
Dudgeon (1908)	100	63
Seedorff (1924)	99	67
Winslow and Winslow (1908)	70	61
Winslow, Rothberg and Parsons (1920)	68	47

The reaction is not a constant one and a strain which has been found negative may, on retesting, liquefy the gelatin (Julianelle, 1922; Dudgeon and Simpson, 1928).

Growth in milk. The changes which occur are variable. Winslow, Rothberg and Parsons (1920) give typical figures for 172 strains :

67 strains showed acid formation and clotting with subsequent liquefaction of the clot.

60 showed acid formation (usually), and clotting without subsequent liquefaction.

7 showed no appreciable change in the reaction.

In 22 the milk became alkaline and the casein was liquefied.

In 16 the milk became alkaline and the casein was not liquefied.

The property of liquefaction of casein is not closely correlated with that of gelatin liquefaction.

Action on proteins. Grown on coagulated serum or egg the colonies form slight depressions in the medium, indicating some digestion of the

albumen. On coagulated egg medium to which neutral red has been added they are crimson, indicating acid formation. Some strains planted in plasma in a slide cell digest the fibrin clot around the colony, especially when the blood has a low antitryptic power (Wright, 1923). It has already been shown that casein is digested by many strains. In peptone broth there is a rise in the amino-acid content, commencing in 24 to 48 hours and reaching its maximum in 4 to 6 days (Rosenthal and Patai, 1914; Julianelle, 1922). This rise is said to be greater with the more virulent cultures (Rosenthal and Patai, 1914). Ammonia is produced simultaneously with the amino-acids. Indole is not produced.

Action on nitrates. Nitrates are reduced to nitrites by almost all strains (Gordon, 1906), confirmed by Winslow, Rothberg and Parsons (1920).

Action on carbohydrates. Staphylococci ferment a variety of carbohydrates and polyatomic alcohols with the formation of acid. Gas is never produced. In broth containing 0·5 per cent. glucose with an original reaction of pH 7·4 practically all strains, whether isolated from air or from human lesions, reach their maximum acidity of pH 4·2 to 4·6 in 24 hours (Julianelle, 1922). Gordon (1905, 1906), who first studied this question, showed that great differences existed between different staphylococci in their fermentative capacities and he attempted to classify them by their action on various sugars together with other characters. Table II gives the more important of his results.

TABLE II.

Acid production by staphylococci in various sugars.

	Maltose	*Lactose*	*Glycerin*	*Mannitol*
Common pyogenic staphylococci	+	+	+	+
Common skin ,,	+	+	+	—
Common scurf ,, A	—	—	—	+
Common scurf ,, B	—	—	—	—

There were many strains, however, which gave anomalous results and a classification based merely on these characters was unsatisfactory. This question is further discussed in connection with classification (p. 23). The most important fermentation is that of mannitol; for whereas the common pyogenic staphylococci practically all ferment this substance it is not fermented by *S. epidermidis* (Gordon, 1906, confirmed by Hine, 1922, and others). Winslow, Rothberg and Parsons (1920), however, state that none of the staphylococci produce acid in mannitol broth. The different indicators used probably account for this discrepancy.

Vitality.

The staphylococcus is a hardy bacterium and resists adverse influences well. It is killed by exposure to moist heat at 60° C. for 1 hour. It maintains its vitality in the dry state for some months. In a protein medium free from fermentable carbohydrates it remains alive for months and sometimes years.

The white *epidermidis* type tend to die out more rapidly than the *pyogenes* type (see p. 14). Robertson's meat medium is one of the best for maintaining cultures alive. Its resistance to antiseptics is variable. It is less easily killed by compounds of the phenol group than are *S. pyogenes* or *B. coli*, but on the other hand it is very susceptible to the action of some dyes, especially those of the triphenylmethane group, such as gentian violet (Churchman, 1912). Staphylococcal growth in broth is completely inhibited by the presence of so little gentian violet as 1 in 2,000,000. In human blood, however, growth occurs in a concentration of gentian violet of 1 in 250,000. It is very sensitive to hydrogen peroxide.

The concentration of chemicals completely inhibiting growth in human blood is as follows :

Phenol	1 in 300
Mercuric chloride	1 ,, 2,000
Eusol	1 ,, 2
Chloramine T	1 ,, 300
Iodine	1 ,, 400
Formalin	1 ,, 300
Potassium permanganate	1 ,, 800
Quinine sulphate	1 ,, 400
Sodium oxalate	1 ,, 400
Potassium cyanide	1 ,, 600
Novarsenobillon	1 ,, 10,000
Brilliant green	1 ,, 2,000
Acriflavine	1 ,, 40,000
Gentian violet	1 ,, 125,000

Distribution.

Outside man and animals. Staphylococci are to be found always in the air and dust of houses and inhabited areas. The majority of these are the non-pathogenic types. The more pathogenic varieties seem unfitted for a saprophytic existence and their distribution is mainly confined to man and the higher animals and their immediate surroundings. In the open air Gordon (1906) found no staphylococci similar to those occurring on human skin and scalp, but these were present in closed rooms after human occupation. In bath water after a normal individual had bathed some 1,000 to 100,000 skin staphylococci per c.cm. were present.

In man. Staphylococci are especially associated with the skin and skin lesions. They are constantly present in the horny layer and in the cutaneous glands. The majority of the cocci give white colonies, but yellow or orange varieties are also of frequent occurrence. They are constantly present also in the anterior nares, the external auditory meatus and the anterior part of the urethra, while they are frequently found without obvious disease in the mouth, posterior nares, and stomach, and less frequently in the intestine.

Pathogenicity.

In man. Staphylococcus is the infecting agent in many diseases, some trifling, some severe. In boils, carbuncles, acute mammary abscesses, deep whitlows and osteomyelitis it is the primary and usually the only infection. It may be the primary infection in many pustular skin infections. In these conditions the cocci are usually of the *aureus* variety, but white strains are not uncommon. As a secondary infection it occurs in any skin lesion involving loss of continuity of the epidermis, in acne, impetigo, eczema and many other skin lesions, also in nasal and post-nasal catarrhs and in broncho-pneumonia, especially post-influenzal. During the 1918–19 influenza epidemic staphylococcal broncho-pneumonia was common in some districts, notably Egypt, Malta (Patrick, 1923), and some camps in America (Chickering and Park, 1919). It is a common secondary invader of the genito-urinary tract, and is often found in chronic urethritis, prostatitis and cervicitis. It may be present in large numbers in the urine in cystitis and pyelitis, and is then frequently associated with the presence of renal calculi.

It may be found in the circulating blood in acute osteomyelitis and other acute infections, and more rarely in malignant endocarditis. Injury to the bones or to the heart valves followed by injections of staphylococci has been found to determine infection of the injured parts.

White varieties have frequently been isolated from malignant growths and may be responsible for some of the inflammatory symptoms associated with these.

Staphylococci have been isolated from pemphigus neonatorum, arthritis, malignant growths and other conditions and have been given names associating them with the disease. There seems no justification for the retention of these names, as the cocci are identical with those isolated from the skin in other conditions.

In animals. Staphylococci are commonly found in warm-blooded animals both in health and in suppurative conditions, especially of the skin. The lesions produced are essentially the same as in man.

S. pyogenes is pathogenic for most of the higher animals. Of the laboratory animals, the rabbit is the most susceptible, and is the one usually chosen for experiment. Mice, especially the Japanese white mice, are also susceptible, while guinea-pigs, rats and dogs have a relatively higher resistance. In the rabbit subcutaneous inoculation induces a localized inflammation which may proceed to abscess formation and usually heals up rapidly. The infection may, however, become generalized (see intravenous inoculation). Intraperitoneal injection is followed by a purulent peritonitis which is rapidly fatal. Intravenous inoculation induces a pyæmic condition and the rabbit becomes emaciated and dies after an interval of three days or more, varying with the dose and the virulence of the culture. The cocci lodge in various parts of the body and give rise to small abscesses, especially in the cortex of the kidney,

but also in the heart and voluntary muscles and not infrequently in other tissues and organs. Sometimes the pyæmic condition is accompanied by suppurative arthritis, and some strains have a special predilection for joints, so that when freshly isolated cultures are injected intravenously in small doses (0·02 c.cm. of a 24-hour broth culture) only a suppurative arthritis results, while larger doses (0·1 c.cm.) are followed by pyæmia and suppurative nephritis. There is also (Rodet, 1884) a predilection for the bones, and especially for their most rapidly growing parts. Endocarditis may follow intravenous inoculation in rabbits (Wyssokowitsch, 1886).

Following intravenous inoculation, the cocci are found in the urine after five hours. They are unable to pass through the intact kidney epithelium, and it is only after the epithelial cells have been damaged by their growth that they appear in the urine (Wyssokowitsch, 1886 ; Dyke, 1923).

Cultures rubbed into the skin of the human forearm produce typical furuncles (Garré, 1893).

Different strains vary enormously in their virulence, some killing rabbits in minute doses while others are ineffective in enormous doses. The virulence sometimes persists in culture and sometimes it rapidly diminishes. The *aureus* strains of *S. pyogenes* are usually more virulent than are the *albus* strains, but there are many comparatively avirulent yellow strains and many virulent white ones. *S. epidermidis* is almost without virulence.

Virulent staphylococci obtained from human keratitis when inoculated by scarification of a rabbit's cornea induce keratitis and hypopyon. Such a strain, which has lost virulence by repeated subculture so that it only produced this effect by intracorneal injection and not by scarification, can have its virulence restored by cultivation on a medium containing lysozyme (egg-white or tears) (see Lysozyme, Vol. VI).

Hæmolysin.

On a blood-agar plate the colonies of staphylococci are surrounded by a clear hæmolytic zone. The filtrate of a broth culture, or better the supernatant fluid of a centrifuged broth culture, when incubated with washed red blood corpuscles, causes complete lysis of the corpuscles. Human corpuscles are less sensitive to this hæmolysin than are those of the rabbit or the sheep.

When testing for this hæmolysin it is important to incubate the mixtures of blood corpuscles and culture at 37° C. and then to allow them to stand for some time at room temperature (or in the ice chest). A much higher titre is obtained by this method than by incubation at 37° C. only (Walbum, 1921, confirmed by Biggar, Boland and O'Meara, 1927). In broth at 37° C. the hæmolysin begins to appear on the fourth day and reaches its maximum in 8 to 14 days (Neisser and Wechsberg, 1901). Walbum (1922) has shown that the optimum temperature is 40° C., when

it reaches its maximum in 3 to 4 days. Some strains which at first show no hæmolysis acquire a hæmolytic power after a repeated subculture on blood agar (Julianelle, 1922). It has been held that strains isolated from more serious lesions are more powerfully hæmolytic, and probably this is generally true, but many strains from healthy skin or scalp show as large an area of hæmolysis around the colonies as do those from acute lesions.

Biggar, Boland and O'Meara (1927) have shown that if 24-hour agar cultures are washed off with a small quantity of normal saline and the suspension is immediately centrifuged, then the clear supernatant fluid is hæmolytic to a marked degree. In some cases washed sheep's corpuscles were completely dissolved by a 1 in 250,000 dilution of this fluid after $1\frac{1}{2}$ hours at 37° C., followed by 2 hours at room temperature. The lysin thus prepared can be obtained in a dry form, when it is soluble in water and salt solution, but insoluble in alcohol, ether, acetone or chloroform. Biggar, Boland and O'Meara found that the hæmolysin prepared in this way was reduced in titre by about one half when heated to 60° C. for half an hour, and there was little further reduction even after boiling for the same time. This differs somewhat from the results of Neisser and Wechsberg (1901) and others, who found that in broth filtrates the hæmolysin was destroyed by heat at 56° C. for 1 hour. Probably the discrepancy is due to the comparatively high concentration of the hæmolysin in the fluid prepared by Biggar's method.

An antistaphylolysin exists normally in serum, especially in the serum of horse and man. By injections of staphylolysin into a rabbit an antibody can be produced which neutralizes the hæmolysin of all staphylococci.

Orcutt and Howe (1922) have produced evidence to show that in some cases the hæmolysis by staphylococci is associated with the presence of a fat-splitting ferment. Lipolytic ferments had previously been demonstrated in staphylococcal cultures (Eijkman, 1901 ; Wells and Corper, 1912).

Leucocidin.

Van de Velde (1894) found that in the pleural exudate of rabbits following injection of virulent staphylococci there was marked evidence of leucocytic destruction. He was able to show that the destructive element was a soluble toxin elaborated by the cocci *in vitro* as well as *in vivo*, and that the staphylococci grown in serum broth produced this leucocidin. The leucocidin affects not only leucocytes, but also tissue cells (Denys and Van de Velde, 1895). It can be produced also in ordinary broth, appearing in four days and reaching a maximum in about one week, after which it gradually disappears (Neisser and Wechsberg, 1901). It is similar in its properties to bacterial exotoxins and is destroyed by heat at 56° C. for half an hour. It can be filtered through a porcelain filter. It is produced by both *aureus* and *albus* varieties, but the amount elaborated by different strains is very variable. It is stated (Neisser and Wechsberg, 1901) that the amount produced varies with the virulence of the strain. Leucocidin development is quite independent of hæmolysin production,

and weakly hæmolytic strains may produce much leucocidin, while strongly hæmolytic strains may produce none (Julianelle, 1922).

An anti-leucocidal substance exists normally in human and horse serum. Denys and Van de Velde (1895) showed that an anti-leucocidin could be produced in rabbits by treating them with pleural exudate containing leucocidin. The same result follows the injection of filtrates of broth cultures containing leucocidin, and it had been shown (Neisser and Wechsberg, 1901) that the anti-leucocidin so produced will neutralize the leucocidin of all strains of staphylococci.

Other Toxic Products.

Cultures killed by heat when injected subcutaneously into animals may induce a local inflammation. Even small quantities of staphylococcus vaccine injected subcutaneously cause in some people a very marked local reaction. Sterile filtrates of some strains of *S. aureus* when injected intradermally into rabbits produce a local inflammation, and with large doses death occurs frequently in 3 to 5 days. Post-mortem examination reveals signs of emaciation only (Parker, 1924). Intravenous injection of the same doses is without toxic effect.

The serum of the animals which have recovered has the power of neutralizing the toxic properties of the filtrates, and in such animals some degree of immunity is obtained to further intradermal injections of the filtrate. The toxic substance is thermolabile, being destroyed by heating to 55° C. for 1 hour. Possibly this is another manifestation of the leucocidin.

There exists, therefore, in staphylococcus cultures a very definite hæmolysin and a cell poison (leucocidin). There are also endotoxins, as evidenced by the results of injections of killed cultures, but the nature of these is obscure.

Pathology of Lesions in Man.

The typical staphylococcal lesion in man is essentially a local one. There is rarely seen the spreading cellulitis which is associated with streptococcal infection. Very soon after the onset there is much emigration of leucocytes around the infected spot. The cellular toxin (leucocidin) produced by the staphylococcus destroys many of the emigrated leucocytes and also the tissue cells, so that in a typical staphylococcal lesion, such as a furuncle, there exists a 'core' of necrosed connective tissue surrounded by a thick pus consisting largely of broken down leucocytes. Acute staphylococcal infection of sufficient magnitude induces a polynuclear leucocytosis.

In acute osteomyelitis the primary infection through which the staphylococci gain entry is usually insignificant, and may even be indiscoverable. In such cases there is frequently a septicæmic condition.

Post mortem in cases of staphylococcal septicæmia, there are found in the tissues and organs and especially in the cortex of the kidney, small abscesses containing many staphylococci.

Resistance.

Man has a very considerable resistance to staphylococci. Although they are constantly present on the skin and in the cutaneous glands, actual lesions are comparatively rare. The natural immunity depends partly on the mechanical protection of the cutis, partly on the ease with which the leucocytes ingest and destroy the cocci after opsonization, and partly on the bactericidal and bacteriolytic substances contained in the cells and secretions. Infection with staphylococci is probably in most cases the result of injury—in some cases, as in whitlows, the injury can be traced, in others, as in boils on the neck round the collar margin, it may be assumed. Staphylococci grow freely in human serum, and a serous exudate on the skin from any cause will become infected with them.

In diabetes mellitus there appears to be a reduction of the resistance to staphylococcus, but the mechanism of this reduction is yet unexplained.

In vitro normal human blood is able to kill from 80 to 95 per cent. of virulent staphylococci provided that the implant does not exceed 2,000 cocci per c.cm. This can readily be demonstrated by incubating infected blood or defibrinated blood in capillary tubes or slide cells. Rabbit or guinea-pig blood possesses much less bactericidal power. This bactericidal power is completely lost if the leucocytes are removed or destroyed.

Immune Substances.

Anti-hæmolysin and anti-leucocidin sera have been produced, but they do not confer any considerable degree of immunity. The same may be said of anti-bacterial sera prepared by immunization of animals with whole cultures.

The administration of a vaccine is followed by a rise in the opsonic content of the serum and by the appearance of agglutinins, precipitins and complement-fixing bodies. It has not been found possible to produce in the rabbit a high degree of immunity to staphylococcus infections.

Agglutinins are normally present in human sera in a titre varying from 1 in 50 to 1 in 1,000. The normal agglutinins present in the sera of different individuals react differently with different strains, and possibly have some relation to past staphylococcus infections. Immunization of rabbits with staphylococcus vaccine is followed by the appearance of agglutinins up to a titre of 1 in 1,000 to 1 in 5,000. These agglutinins react with the homologous and with some other strains, but not with all. This will be further discussed in relation to the classification of the staphylococci.

Precipitins are present in the serum of about 42 per cent. of normal individuals and only in a slightly higher percentage (55 per cent.) in people with known *S. aureus* infections. Patients suffering from serum sickness give stronger precipitin reactions than do infected individuals with antigens prepared from *S. aureus* cultures in peptone broth (Dudgeon and Bamforth, 1925).

Classification.

Rosenbach (1884) classified staphylococci by the colour of the colony into *S. aureus* and *S. albus*, and Passet (1885) added a lemon-yellow coloured type (*S. citreus*). This classification has proved unsatisfactory, and it has been shown that in certain circumstances the cocci may lose their chromogenic power.

Gordon (1906) attempted to classify staphylococci by their action on gelatin, milk, nitrates, neutral red, maltose, lactose, glycerin and mannitol. He showed that, in addition to *S. pyogenes*, which was the usual type isolated from acute lesions, the common staphylococcus of the skin, which he identified with Welch's *S. epidermidis albus*, had distinctive characters, as also had two common types occurring in the scalp. Dudgeon (1908) by similar methods, produced evidence to show that the white and orange pathogenic forms were varieties of a single species (confirmed by Winslow, Rothberg and Parsons, 1920), and Seedorff (1922).

Before this, serological tests had been made. Kolle and Otto (1902) had stated that by agglutination tests with an immune serum the pathogenic types could be distinguished from the non-pathogenic. Walker and Adkinson (1917) found that an *aureus* immune serum agglutinated *aureus* and not *albus* strains and vice versa. Dudgeon and Simpson (1928), on the other hand, using a precipitin test, found that there was no essential difference between the orange and white pathogenic varieties.

Hine (1922) found that simple agglutination tests were insufficient clearly to separate the staphylococci into groups, and substituted absorption tests. By this means he classified the staphylococci into two main types—(1) *S. pyogenes* (mixed orange and white), and (2) *S. epidermidis* (almost all white). *S. pyogenes* was almost a homogeneous group, only 10 per cent. varying from the type, and these exceptions fell into two groups. The *S. epidermidis* strains serologically fell into two large groups. The incidence of these groups were as follows :

	S. pyogenes			S. epidermidis		*Total*
	I	*II*	*III*	*I*	*II*	
Septicæmia	9	—	1	1	2	13
Boils, carbuncles, abscesses ..	13	1	—	1	1	16
Tonsillitis, pharyngitis, rhinitis, otitis	12	1	—	—	—	13
Broncho-pneumonia, empyema, sputum, osteomyelitis	10	—	—	—	—	10
Various septic conditions	6	—	—	—	2	8
Heart blood p.m.	4	1	—	—	—	5
Urine	1	—	—	1	1	3
Skin, conjunctiva, &c.	—	1	1	9	2	13
	55	4	2	12	8	81

The *S. pyogenes* types (with few exceptions) ferment mannitol, while the *S. epidermidis* types (with few exceptions) do not.

On the available evidence it would seem that the colour of the colony is useless in classification, except in so far as the orange staphylococci are, as a rule, more active in many respects, e.g. liquefaction of gelatin, fermentation of sugars, virulence, and possibly also in the production of hæmolysin and leucocidin. There is some evidence also that the chromogenic power is not constant, and that the *aureus* strains may in certain conditions lose this property. The liquefaction of gelatin is an untrustworthy test, as it may vary from time to time with the same strain. This property also, as well as the changes produced in milk and the fermentation of sugars, may differ in strains which are serologically identical. The fermentation of mannitol may be used as a rough test to distinguish between *S. pyogenes* and *S. epidermidis* groups, the former fermenting and the latter failing to ferment this substance. Serologically Hine's classification by means of the absorption test into *S. pyogenes* and *S. epidermidis* seems to be the most satisfactory. The former group consists almost entirely of one serological type, there being few exceptions, but the latter has at least two commonly occurring serological types, and Gordon's work on the cocci of the scalp makes it likely that other types may be differentiated. It must not be forgotten, however, that it has been shown that an antiserum prepared to the hæmolysin or the leucocidin of one strain will neutralize those of all other strains tested, and it is possible that the staphylococci are really one homogeneous group, the members of which, while they may have in different circumstances acquired or lost certain properties, are yet essentially the same.

Treatment.

Passive immunity by means of antibacterial serum has been attempted in many staphylococcal infections without marked success. Better results have attended active immunity and it was in connection with such infections that Wright (1902 and 1909) introduced vaccines for therapeutic purposes. He showed that the inoculation of killed cultures was followed by a rise in the phagocytic power of the patient's blood and by a marked improvement in the clinical condition of patients suffering from furunculosis, sycosis and acne. Since Wright's original publication vaccine treatment has become general in staphylococcal infections. The vaccine is not very toxic and large doses (100 to 2,000 millions) can be administered with little risk of a general reaction when inoculations are made subcutaneously. Comparatively small doses, however, injected intravenously, are followed by fever and malaise. In some individuals there is a very considerable local reaction at the site of inoculation and sometimes moderate-sized doses induce a marked focal reaction, leading, in the case of a furuncle or carbuncle, to rapid breaking down of the lesion. As in such cases there is little risk of the infection becoming generalized, this focal reaction may be used to hasten the healing of indolent lesions.

In *S. pyogenes* infections stock vaccines have been found to be practically as efficient as autogenous ones, as would be expected from a consideration of the serological results described above. The vaccines may be used in localized infections, such as furuncles, or in more serious infections attended with grave constitutional disturbance. In the latter case the amount administered should be small, say 5 to 20 millions, and the doses may be repeated at intervals of 1 to 3 days. The immunity produced by infection or by vaccines is shortlived and re-infection may take place in a few weeks, although many patients following a course of vaccine treatment are free from infection for years.

Besredka (1923) has claimed that by intradermal injections of filtrates of staphylococcal cultures ('antivirus') or better still, by applying dressings soaked in such filtrates to the epilated skin of animals, a high degree of local immunity is obtained. If the filtrate is injected intravenously no immunity is obtained, and if subcutaneously local immunity only develops to a lesser degree. The 'antivirus' is made by filtering an 8- to 10-day-old broth culture of a virulent staphylococcus, and Besredka states that such a filtrate has a specific inhibitory effect on the growth of staphylococci. This method of using dressings soaked in staphylococcal filtrates has been applied to man in the treatment of superficial staphylococcal infections. Besredka's claims have been subjected to much criticism. Gratia (1923) showed that dressings soaked in plain broth gave as good local immunity as those soaked in staphylococcal broth filtrate. (Contradicted by Urbain, 1924, and others.) Mallory and Marble (1925) confirmed Gratia's work and Dold and Müller (1928) were likewise unable to confirm Besredka, whose claims cannot yet be considered to be established.

Manganese and tin preparations have been used in treatment, and they may have had some clinical success, although no adequate reason why they should be successful has been forthcoming. Other chemotherapeutic measures in the shape of intravenous injections of gentian violet and mercurochrome have been used extensively, especially in America, and some good results have been published. It has been shown (Colebrook and Hare, 1927) that mercurochrome in a greater concentration than can be obtained by intravenous injection has no inhibitory action on the growth of staphylococci in blood. Gentian violet which, in any concentration which it is possible to obtain in the body, acts only slowly on staphylococci, can be shown to disappear rapidly from the circulating blood after intravenous administration. It is unlikely, therefore, that these drugs can have any direct bactericidal action on the staphylococci in the body in a generalized infection. There is no evidence of any indirect action.

Other Cocci resembling Staphylococci.

At different times various names have been applied to cocci which, although morphologically identical with staphylococci, yet differed from the type in certain particulars. The characters on which the classification

has been made, however, are in most cases insufficient to warrant a differentiation from the staphylococci or the application of a specific name. Hucker (1924) deals with this subject and cites 32 names which have been applied to *S. aureus* and 48 to *S. albus*. The name *M. ureæ* has been given to a variety of white staphylococcus which differs from the type only in being able to break down urea and use it as its only source of nitrogen. Serologically it has affinities to some of the other white staphylococci (Hucker, 1924).

In addition to these which really are staphylococci there are varieties of micrococci widespread in nature which on morphology alone might be confused with this group. These are non-pathogenic and frequently occur as air contaminations on culture plates. The elements are as a rule larger than the typical staphylococcus and are not so regular in size. The optimum temperature of growth is generally around 25° C., and the rate of growth is usually slower than that of staphylococci although ultimately the colonies may reach a large size. The colonies may be white, red, or more frequently, bright canary yellow in colour. Many of these cocci are readily dissolved by human tissues and secretions, and one type has been isolated (*M. lysodeikticus*) which has this property to such a degree that a 1 per cent. solution of human tears, nasal mucus or sputum will completely dissolve a thick suspension in a few seconds (Fleming, 1922).

Micrococcus tetragenus.

Gaffky (1881) described this coccus, which is characterized by an arrangement in tetrads. In specimens from the body the cocci are large, often flattened on their opposing surfaces, and each group or tetrad is surrounded by a large capsule. In culture the cocci are irregular in size ; in young cultures the individuals are, on an average, larger than staphylococci, and the majority are arranged in tetrads ; the capsule, however, is not evident except in cultures in serum. In older cultures the cocci are mostly smaller and the arrangement is less definite, there being many single cocci, pairs, and small irregular groups. *M. tetragenus* is strongly Gram-positive and stains readily with all the ordinary bacterial stains.

Cultural characters. M. tetragenus is an aerobe and facultative anaerobe. It grows well on all the ordinary media. The optimum temperature for growth is 37 to 39° C., and it ceases below 15° C. or over 42° C. On agar the colony is round, regular and greyish white. It is smaller and less opaque than a staphylococcal colony. On blood agar it is surrounded by a green area like that produced by *Streptococcus viridans*. In broth there is at first an even clouding of the medium, and later a copious viscous deposit. Gelatin is not liquefied. Different strains vary one from the other in their sugar fermentations. The colony of the typical *M. tetragenus* is not coloured, but strains have been described having a pale yellow or a definitely orange colour (Chauffard and Ramond, 1896).

Pathogenicity for animals. *M. tetragenus* is very pathogenic for the white mouse, less so for the guinea-pig, while rabbits and grey mice are relatively immune. According to the virulence of the culture, subcutaneous injection into the white mouse or guinea-pig is followed by induration, necrosis, or abscess formation which leads to a septicæmia of variable intensity, and death of the animal; in the case of the mouse in 2 days or more, and in the case of the guinea-pig in about 10 days. The blood, especially that of the mouse, contains many capsulated tetrads. Intraperitoneal injection is followed by a membranous exudation or a hæmorrhagic or purulent effusion leading to septicæmia. The ingestion of cultures has been followed by septicæmia (Teissier, 1896).

Pathogenicity for man. This microbe is frequently found in the sputum in cases of chronic tuberculous or other infection. It may be present in large numbers, but it is seldom that it plays other than the role of a secondary invader. It has been described as occurring in meningitis, and has been isolated from the blood in cases of septicæmia. It has been said to have been isolated from dental abscesses, from the mouth, nose, tonsils and many other parts of the body, but frequently the only evidence for its occurrence has been the presence of tetrads in films or cultures, and there is no doubt that there has been some confusion between *M. tetragenus* and staphylococci. Its natural habitat seems to be the respiratory tract, where it may exist without any obvious pathogenic effect, or it may be responsible for minor catarrhal inflammations. Rarely does it invade the body, giving rise to septicæmia or meningitis. It has been described as occurring in spontaneous abscesses in domestic animals.

Immunization of rabbits with vaccines of *M. tetragenus* is followed by the appearance of agglutinins in the rabbit's serum. Hucker (1924) has shown that there is some cross agglutination and complement fixation between this organism and some of the white staphylococci.

M. tetragenus may be distinguished from the typical staphylococcus by the presence of a capsule in material from the body (sputum, &c.), by its greater irregularity in size, and by its pathogenicity for the white mouse, in which capsulated tetrads are found in the blood after death. The fact that there is serologically some relationship with staphylococcus makes it possible that *M. tetragenus* is merely a specialized member of the staphylococcus family.

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CHAPTER II. THE STREPTOCOCCI.

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History.

By W. Bulloch.

The bacteriology of streptococcus opens with the investigations on erysipelas which had gradually come to be regarded by clinicians as an infective process. R. Koch (1881) found in all cases of this disease one and the same chain form of coccus, and Fehleisen (1881, 1882) not only confirmed this but succeeded (1882, 1883) in growing it in pure culture. He further showed that subcultures produced typical erysipelas in five human beings. For a considerable period Fehleisen's *Streptococcus erysipelatis* was deemed to be specific, but by degrees it was recognized that it could not be differentiated with certainty either by form or culture from the streptococcus which Rosenbach (1884) named *S. pyogenes*. The belief in the specificity of *S. erysipelatis* was abandoned after Koch and Petruschky (1896) showed that typical clinical erysipelas can be produced in man by streptococci from such purulent processes as peritonitis.

While this was going on a very large literature was accumulating on the production of disease in man and animals by cocci in chains. Some of the lesions were purulent, others were non-suppurative and often of special clinical types such as endocarditis, and puerperal fever. In general, streptococcic lesions, as was first shown by Rosenbach, were found to be of a spreading rather than of a focal type. Many different streptococci were named but there was often a doubt whether they represented different species. Differentiation on morphological and cultural grounds was attempted by v. Lingelsheim (1891) with only partial success. P. H. Hiss (1902, 1901–5) introduced differential tests by means of fermentation reactions on carbohydrates, and these were extended by M. H. Gordon (1905) and Andrewes and Horder (1906). H. Schottmüller (1903) introduced a method of differentiation depending on the growth of streptococci on media containing 'whole' blood. The production and properties of anti-streptococcic serum were described by Marmorek (1895) and Aronson (1896).

The Streptococci of the Human Body.

J. W. McLeod.

As early as 1874 Billroth published a considerable volume in which he attempted a morphological classification of the bacteria found in putrefying tissues and in discharges from the animal body. It is by him that the name Streptococcus was introduced (see Vol. I). He classified the coccal forms as: Monococcos, Diplococcos, Streptococcos and Gliacoccos. He noted the presence of 'streptococcos' in 50 per cent. of the cases of erysipelas which he examined; but he argued at length to show that there was no satisfactory evidence for supposing that the inflammatory conditions examined were due to the bacteria present.

It is evident from an account of a discussion at the Académie de Médécine (1879) and from the treatises published by Doleris (1880) and Duclaux (1882) that Pasteur was convinced that a 'micrococcus *sous forme de chapelets*' played an important part in the causation of puerperal fever, and could be cultivated from the blood in some cases, but he does not appear to have demonstrated its pathogenicity by animal experiment. The first experimental demonstration of this was that of Koch (1878), who, starting out with decomposing blood containing a bacillus and a streptococcus, killed mice in 24 hours, the bacillus being apparently mainly responsible for the rapid death of the animal; by using field-mice, however, which were resistant to the bacillus, he succeeded in obtaining pure streptococcal infections characterized by spreading tissue necrosis or cellulitis, multiplication of chain cocci in the tissues, and death in 3 days. The streptococci are clearly figured in his illustrations.

The first complete proof of the connection of cocci with disease in man we owe, however, to Ogston (1880, 1881, 1882 and 1883), who demonstrated in a large series of cases the presence of chain cocci in human lesions, and reproduced similar lesions in experimental animals both with injections of pus and with cultures of cocci obtained in sterile eggs after many unsuccessful attempts in other media, and Fehleisen (1882), applying Koch's (1881) new cultural methods, put the connection of streptococci with erysipelas beyond all possible criticism. Fehleisen's work included the production of erysipelas in a woman suffering from generalized sarcoma by injection of a streptococcal culture.

Rosenbach (1884) introduced the term *Streptococcus pyogenes* to indicate a form responsible for suppurations, but distinct from the streptococcus of erysipelas. The criteria by which he distinguished these two forms—appearances of gelatin and agar cultures—have not been found adequate by subsequent observers (Fraenkel, 1889; Lingelsheim, 1891). The idea, however, which must have influenced Rosenbach, i.e. that streptococci producing forms of disease which are clinically distinct must differ in some respects, has stimulated innumerable workers to attempt

differentiations in the streptococcal group. Before proceeding to discuss these, however, it is desirable to survey the development of our further knowledge with regard to the pathogenic role of the streptococci.

Puerperal fever. Although, as already indicated, the connection of streptococci and puerperal fever appears to have been definitely suggested by Pasteur, it is to Fraenkel (1884) that we owe the first clear demonstration of the bacteriology of the different types of puerperal infection. He showed the predominating role of the streptococcus in those types characterized by rapid spread of the infection through the subserous layer of the peritoneum, and proved the pathogenicity of these streptococci for mice and rabbits, and lastly, their identity with the streptococci of erysipelas. It was also in connection with infections of the female genital organs that the existence and pathogenic significance of anaerobic streptococci was first clearly indicated (Veillon, 1893 ; Menge and Krönig, 1899).

Septicæmia and endocarditis. In the earlier investigations blood culture as we now understand it was not attempted, at most drops of blood drawn from the finger, &c., were examined, mostly with negative results, and emphasis was laid chiefly on the local lesion and the toxæmia resulting from absorption. The investigations on endocarditis were limited to examination of the vegetations at autopsy. The streptococcus was either noticed amongst other bacteria and found, like some of them, to be capable of producing acute endocarditis when injected intravenously to a rabbit whose heart-valves had been injured previously (Wyssokowitsch, 1886 ; Weichselbaum, 1887), or its presence was not recorded at all (Fraenkel and Saenger, 1887). In 1894, however, Grawitz and Sittmann independently drew attention to the fact that, with the exception of a few conditions such as anthrax and relapsing fever, bacteria did not occur in the blood in sufficient numbers to be observed by direct microscopic examination, and that for demonstration of blood infection venipunction and withdrawal and cultivation of considerable amounts was necessary in the case of most septicæmias. Even with these improved methods the importance of the streptococcus in endocarditis was not fully realized (Kuhnau, 1897 ; Canon, 1905) until Lenhartz (1903) began to differentiate clearly between acute septic and chronic ulcerative endocarditis, supported by Schottmüller's (1903) work on differentiation of streptococcal types.

Scarlet fever and angina. Although it was not till quite recently, owing to the results obtained on adoption of man for experimental investigation by the Dicks (1916, 1921, 1924) that the streptococcal causation of scarlet fever was generally accepted, the idea of a close association between streptococci and scarlet fever is an old one. Thus, Fraenkel and Freudenberg (1885) found streptococci present in all the viscera in three fatal cases, and thought it not unlikely that the body was always invaded by streptococci in this disease. They concluded, however, that its role was that of a secondary invader, since (1) the

strains obtained could not be distinguished in any way from *Streptococcus pyogenes*, and (2) they could not be cultivated from the skin even when portions were excised during life. This opinion of the part played by the streptococcus in scarlatina was shared by Raskin (1889), Böhm (1891) and others, and remained till quite recently the most general view with regard to the question. Kurth (1891), although not committing himself very definitely, suggested the possibility of differentiating the streptococci of scarlet fever by the character of their growth in broth and by their high virulence for mice. Babes (1887), however, was struck by the constant presence of streptococci in the kidneys in a local epidemic characterized by a special tendency to nephritis, and he considered that the streptococcus was probably responsible for the disease, and Baginsky and Sommerfeld (1900) defined the role of the streptococcus in scarlet fever with remarkable precision as we know it to-day. They considered scarlatina to be due to streptococcal invasion of the throat with production of the general symptoms by absorption of toxin and a tendency to terminal streptococcal invasion of the viscera in fatal cases. Only the fact that they had no criterion by which they could distinguish scarlet streptococci from others led them to express their conclusions tentatively. They believed it probable, however, that some criterion for making the distinction would ultimately be found.

Kurth (1889) noted that *Streptococcus pyogenes* was constantly present in the throat in cases of inflammation of the tonsils and absent in normal throat ; his series of cases was a small one, however, and he quotes the finding of others that *S. pyogenes* is present in 4 to 8 per cent. of normal throats. Hilbert (1899) maintained that the occurrence of streptococci in the mouth was first noted by Biondi in 1887. His own observations led him to conclude that streptococci were constantly present in the mouth, that many of those from normal throats were pathogenic to mice, and that therefore there was no evidence for the association of streptococcal infection and sore throat. It was left, therefore, to the more modern work in which better methods of differentiating were used to clear up the role of streptococci in inflammation of the tonsils.

Rheumatism and arthritis. Although it cannot be said that an ætiological connection between the streptococci and acute rheumatism has been fully established, yet an account of the development of our knowledge with regard to the pathological role of the streptococcus would not be complete without some reference to it. Dana (1894) noted the presence of a diplococcus in the meninges in a case of chorea, and Apert (1898) and Triboulet (1902) noticed the association of streptococci with cases of rheumatism ; but it was the work of Westphal, Wassermann and Malakoff (1899) in Germany and that of Poynton and Paine (1900) in this country, which first concentrated attention on the idea that acute rheumatism was a streptococcal infection. These supported their other observations by the experimental production of arthritis in the rabbit. A careful scrutiny of Poynton and Paine's work shows, however, that

their rabbits for the most part developed arthritis in the course of a fatal infection induced by very large intravenous doses of streptococci. The relationship of such a condition to acute rheumatism is far from clear, but the work of these observers at all events served to emphasize the importance of the streptococcus as a cause of arthritis. In this connection it is interesting to note that Lenhartz (1903) draws attention to the facts that in streptococcal infections metastasis is specially liable to occur in the joints, and with staphylococcal infections, in the kidney.

Classification and Differentiation.

By Cultural and Biochemical Tests.

The criteria of differentiation amongst streptococci which still influence our opinions may be said to originate with Schottmüller's (1903) classical work. He first used the fresh blood-agar plate and made a classification into three main types according to the appearances of the growths on this medium : Type 1, *Streptococcus longus pathog.* sive *erysipelatos*, including all highly pathogenic strains and characterized by the development of clear colourless haloes around its colonies ; Type 2, *Streptococcus mitior* sive *viridans*, including mainly strains derived from the respiratory or digestive tracts and of low pathogenicity. These may show narrow rings of clearing around their colonies on agar of low blood content or prepared with blood unusually susceptible to disintegration, but are chiefly distinguished by their tendency to produce a green discoloration of the blood-agar in their immediate vicinity. Type 3, *Streptococcus mucosus*, of much rarer occurrence than the foregoing and distinguished by its abundant mucoid colonies, and by the well-defined capsule seen in microscopic preparations even from cultures on solid media.

An obvious weakness in this scheme was that the Pneumococcus was not very clearly differentiated from either Types 2 or 3 without subsidiary tests, such as virulence for mice. E. Fraenkel (1905), however, pointed out that the virulence test was not always reliable for differentiating *Pneumococcus* from *Streptococcus viridans*, and that this differentiation could be best effected with litmus-nutrose-agar, on which the former grew poorly and the latter developed a bright red colour.

It was Gordon (1904) and (1905), however, who first extensively applied fermentative and other biochemical tests to the differentiation of the streptococci. Gordon's tests were the clotting of litmus milk in 3 days, the reduction of neutral red in broth cultures within 48 hours under anaerobic condition and the fermentation of seven sugars. The strict application of these tests led to the differentiation of a rather bewildering number of types. Andrewes and Horder (1906) took up Gordon's tests and examining a large series of streptococci indicated the existence of half a dozen main groups. These were :

(1) *Streptococcus equinus*. The streptococcus of horse dung, characterized chiefly by inability to ferment lactose or mannitol or to clot milk.

(2) *Streptococcus mitis.* Common in mouth and fæces and characterized by good growth in gelatin at 20° C. and reduction of neutral red.

(3) *Streptococcus pyogenes.* Common in suppurative processes, characterized by hæmolytic activity and inability to reduce neutral red.

(4) *Streptococcus salivarius.* The common mouth streptococcus, characterized by clotting of milk, reduction of neutral red, and frequently by fermentation of raffinose.

(5) *Streptococcus anginosus.* Not distinguished from *S. pyogenes* except by reduction of neutral red, and clotting of milk.

(6) *Streptococcus fæcalis.* The common bowel streptococcus, which is characterized by H_2S production and by the activity of its fermentations.

Their chief results are summed up in Table I.

TABLE I.

	S. pyogenes.	*S. salivarius.*	*S. anginosus.*	*S. fæcalis.*	*Pneumococcus.*
Clotting of milk	−	+	+	+	±
Reduction neutral red	−	±	±	+	−
Growth in gelatin at 20° C. ..	+	±	±	+	−
Morphology ..	Long chains.	Short chains.	Long chains.	Short chains.	Short chains.
Mouse pathogenicity	+	−	+	−	+
Fermentation of lactose ..	+	+	+	+	+
Fermentation of saccharose ..	+	+	+	+	+
Fermentation of raffinose ..	−	±	−	−	+
Fermentation of inulin.. ..	−	−	−	−	±
Fermentation of salicin ..	±	−	−	+	−
Fermentation of coniferin ..	−	−	−	+	−
Fermentation of mannitol ..	−	−	−	+	−

A good deal of criticism has been brought forward in regard to both of these schemes of classification. In Germany, the view has been expressed repeatedly that hæmolytic streptococci can be changed during subculture to green-forming strains and vice versa, and hence that there is really only one type of streptococcus, subject to variations according to conditions of preservation and culture (Zoeppritz, 1909 ; Kuczynski and

Wolff, 1920; Philipp, 1924; Demme, 1925). Morgenroth and some of his collaborators actually extended this idea of ready conversions of streptococcal types to include the Pneumococcus (Morgenroth, Schnitzer and Berger, 1925; Berger and Jakub, 1925). Schottmüller (1924), however, has stoutly maintained his position and points out the absurdity of a contention for the unity of streptococci when anaerobic forms producing foul-smelling gases are well recognized, and when *viridans* strains are readily distinguished from hæmolytic streptococci by their marked sensitiveness to the bactericidal action of defibrinated blood.

The criticism most raised against the work of Gordon and of Andrewes and Horder is that the sugar fermentation or other tests which they employ are not given constantly enough by any one strain of streptococcus to serve as a basis for differentiations. This has been stressed particularly by Ainley Walker (1911) and Ritchie (1908), in this country, and by Buerger (1907) in America, who pointed out that it was only necessary to give a streptococcus a more favourable medium in order to enable it to ferment some sugars which it had not previously fermented.

Since the value of the fermentation of sugars as a basis for the classification of the streptococcus is a rather controversial point, it is necessary to review the literature bearing on it with some care. Less attention appears to have been given to this subject in France and in Germany than in the English-speaking countries. The position taken up by Burnet and Weissenbach (1918), however, probably expresses the French attitude. This is, that, after excluding the pneumococcus and the enterococcus from the discussion as being forms distinct from, although allied to, the streptococci, the remaining forms are classified into two main categories, hæmolytic strains and *viridans* strains, and that immunity reactions support such a division, while they do not coincide with any grouping by sugar fermentation.

In Germany the results described in one of the few papers that have appeared on the subject (Salomon, 1908) diverge so widely from those recorded elsewhere that they cannot be accepted as probable. It is possible that he overlooked the tendency shown by ascitic fluid to turn alkaline on standing (Murray and Ayrton, 1924; McLeod, Wheatley and Phelon, 1927), and that this change in the ascitic fluid used in his media masked the fermentations which he should have observed. Maass (1913) expressed the opinion that there was no correspondence between groupings by sugar fermentation and source or pathogenic importance of streptococci.

It is in North America, however, that most work has been devoted to this subject in the last twenty years. The validity of a streptococcal classification based on sugar fermentations has been most definitely asserted by Holman (1916), who, departing from an initial division into hæmolytic and non-hæmolytic strains, subdivides his strains by fermentation of lactose, mannitol and salicin and thus arrives at 8 hæmolytic and 8 non-hæmolytic types. Hopkins and Lang (1914) have also found

fermentation reactions sufficiently reliable, and so has Lyall (1914), who did not meet variation in their sugar reactions in more than 5 per cent. of his strains. Blake (1917), however, states that the division into hæmolytic on the one hand and *viridans* or indifferent strains on the other by the blood-plate method gives a cleavage more in line with that obtained by agglutinating sera than do sugar fermentations. He sees no object in further classification of the hæmolytic strains by fermentative activities, but would reserve sugar fermentations for further differentiations amongst the non-hæmolytic strains. The same opinion, that there is no correspondence between serological and fermentative classification, is expressed by many independent observers. Kinsella and Swift (1917) examining groupings by agglutination and by complement fixation noted this divergence ; Howell (1918) determining complement fixation did the same, and so did Hamilton and Havens (1919) and Valentine and Mishulow (1921) investigating agglutination. Barnes (1919) compared the sensitiveness of various strains to precipitating sera and found a general correspondence to Holman's groups but some considerable exceptions. Fisk and Burky (1922) found, like Blake, that their serological methods justified a first division into hæmolytic and non-hæmolytic strains, but did not justify further subdivisions by fermentative reactions. More direct criticism comes from Thro (1914) who found that a number of secondary strains picked from separate colonies of a streptococcus varied in their fermentation reactions from time to time, and from Bergey (1912) who did not find it practicable to apply fermentation tests to the recognition of the source of streptococci in water supplies. When we turn to the more recent British work we get similar findings. In the Medical Research Council report on war wounds it is stated that mannitol-fermenting streptococci of *pyogenes* type are not differentiated from non-mannitol-fermenting streptococci by agglutination. The same point is brought out by Gordon's own work (1921). Dible (1921) found Holman's different fermentative types distributed equally in different lesions, erysipelas, cellulitis, puerperal infections, &c. McClachlan (1927) and others conclude that scarlatinal streptococcus strains are not distinguished from other hæmolytic streptococci by their fermentative reactions.

It would appear, therefore, from the foregoing, firstly, that fermentative reactions alone are not a safe guide to the pathological significance of a streptococcus, and secondly, that the majority of those who have investigated the point have failed to establish for the streptococci any such close relationship between antigenic qualities and fermentative activities as exists in the typhoid-paratyphoid group of bacteria. In the opinion of the writer, therefore, an arbitrary classification of streptococci based on sugar fermentations such as that of Holman (1916) is undesirable, but since it has been widely used (Eagles, 1924 ; FitzGibbon and Bigger, 1925 ; Cunningham and Ramakrishnan, 1925) it is given here for purposes of reference in Table II.

TABLE II.

	Lactose.	Mannitol.	Salicin.
S. infrequens	+	+	+
S. hæmolyticus I	+	+	−
S. pyogenes	+	−	+
S. anginosus	+	−	−
S. hæmolyticus II	−	+	+
S. hæmolyticus III	−	+	−
S. equi	−	−	+
S. subacidus	−	−	−

It is possible that in the future further developments in these tests may be devised and may be of value even in differentiating various hæmolytic streptococci, but meantime their chief use appears to be that of subsidiary tests. There is no doubt, however, that used as they were in the work of Andrewes and Horder (1906; Andrewes, 1913), together with other characteristics to establish the existence of different types of streptococci predominant in different areas of the body they have been of value in developing our knowledge of the bacterial flora of the alimentary tract as well as that of the streptococcal group. For example, the definition of *Streptococcus fæcalis* as a special type distinct from the mouth streptococcus was not appreciated in Schottmüller's work, and has only been recently recognized in Germany (Meyer, 1926). This seems to the writer more justifiable than the separation of the bowel streptococci as distinct bacteria under the name of '*Enterococcus*' as is done by the French.

Differentiation and Classification by Serological Methods.

An increasing number of attempts to classify streptococci by serological tests has characterized the bacteriological work of the last few years, but these have dealt almost entirely with the hæmolytic streptococci and will be discussed below (p. 61). Attempts to determine the relationships of the streptococci as a whole based on such tests have not been numerous and have given rather conflicting results. In the work of Durand and Sedallian (1923) who distinguished six groups of streptococcus by agglutination methods so little detail about the nature of the organisms examined is given that its significance is obscure. Kinsella (1918) found that hæmolytic streptococci, as judged by complement-fixation studies, were more nearly related to one of two main groups of non-hæmolytic streptococci distinguished by Swift and Kinsella (1917), the one containing a high percentage of strains fermenting salicin. Howell (1918), however, found that complement fixation was better with hæmolytic streptococcus antigen independently of whether hæmolytic or non-hæmolytic streptococci had been used to inject the animal whose serum was to serve as antibody. But Nakamura (1923) emphasizes the

doubtful value of complement-fixation reactions in the streptococcal group, maintaining that they depend more on the constituents of the medium than on the bacteria and that irregularities may therefore be expected. Some support for this view is derived from Sherman's (1919) observations on hæmolytic streptococci from the throats of dogs. He found that on immunizing a chicken with a *pyogenes* strain from this source he obtained a serum which agglutinated the homologous strain more strongly than others and also had most opsonic effect on it, but gave better complement fixation with another strain, a *Streptococcus anginosus*. Tricoiae (1920) states that an antiserum prepared with enterococcus gives complement fixation with that organism and with pneumococcus but not with streptococcus. There is not much that is convincing in all this, but when we turn to the line of observation suggested by Schottmüller (1924), i.e. the sensitiveness of streptococci to the natural bactericidal agencies of the body, we find reaffirmed the only conclusion that emerges with some consistency from the work on serological classification. That is that a differentiation into hæmolytic and non-hæmolytic is important. Schottmüller as already stated found that the so-called *viridans* strains were more readily killed off by normal serum than hæmolytic streptococci, and in this contention he is supported by Lehmann (1926) and by Nakamura (1923), who found that *viridans* strains were clearly differentiated from hæmolytic ones by their sensitiveness to the bactericidal action of serum leucocyte mixtures.

Meaning of the terms 'Hæmolytic' and 'Viridans' as applied to Streptococci.

Since, as may be gathered from the foregoing, the differentiation between hæmolytic and *viridans* strains is important, and since there is a good deal of confusion of ideas with regard to the meaning of these terms, it will be well at this stage to define and explain them. In a recent contribution on streptococcal hæmolysin Cesari, Cotoni and Lavalle (1927) maintain that the hæmolytic activity of the streptococcus was first noted by Knoor in 1893, who observed evidence of hæmolysis in infected animals. Our knowledge of the hæmolytic activity of the highly pathogenic types of streptococci was first developed, however, in France (Besredka, 1901 ; Marmorek, 1902 ; Breton, 1903), and the observations of these workers were made either on fluid cultures and their filtrates or on infected animals. But it was Schottmüller's (1903) introduction of the blood-agar plate which led to the differentiation that we are discussing, to a much wider use of the hæmolytic criterion and probably also to a good deal of the confusion that exists in regard to this subject. The appearance of a streptococcal culture on a blood-agar plate which is accepted as evidence of hæmolytic activity is the development of clear, colourless, transparent haloes in the opaque medium in the immediate vicinity of the bacterial colonies. Now this is not simply hæmolysis, it is hæmolysis plus destruction or removal of blood-pigment, and it apparently may occur although the coccus concerned does not readily secrete a hæmolytic

toxin. At all events discrepancies between the plate method and the tube method of observing hæmolysis have been noted by several workers (Lyall, 1914 ; Mishulow, 1921 ; Cumming, 1927). In the latter method a small quantity of the fluid culture 15 to 20 hours old is added to 1 c.cm. of a 5 per cent. suspension of washed blood in 0·85 per cent. salt solution, and the result is read after 1 to 2 hours' incubation at 37° C.

The usual discrepancy is that strains appearing to be hæmolytic on the plates are not found to produce hæmolysis when their fluid cultures are added to blood suspensions. The question therefore arises whether such discrepancies are due to the fact that the blood-plate is a more delicate or a less reliable method for determining hæmolytic action. If by hæmolysis is meant any kind of destruction of the blood corpuscles, then the plate method is certainly the most delicate, but if a specific action of a toxin which has a high affinity for and disintegrating effect on some constituent of the limiting membrane of the red cell is meant, then there is little doubt that the plate method is less reliable, for in it the blood corpuscles are brought into contact with all the products of bacterial metabolism in a high concentration and over a considerable period of time.

Van Loghem (1913) and Baerthlein (1914), working with cholera and cholera-like vibrios, emphasized the importance of distinguishing between hæmolysis and hæmodigestion on blood-agar plates, and Boxer (1906) pointed out that complete destruction of the blood corpuscles was characteristic of plate hæmolysis, but not of hæmolysis by the tube method. Lyall (1914) maintained that ' hæmolytic ' effect on plates might be due to acid, and indistinguishable from true hæmolysis, and Davis (1917) has shown that specific streptococcal hæmolysis is independent of acid, since it occurs in a blood glucose agar plate to which powdered calcium carbonate has been added to neutralize any acid formed. Cumming's (1927) observations are specially interesting in this connection—he found all streptococci from frank suppurative lesions hæmolytic equally on human blood-agar plates and on rabbits' blood either by plate or tube method of observation ; but a number of strains isolated from sputum were hæmolytic only on human blood-agar plates. For these he suggests the description pseudo-hæmolytic.

There are at least three factors which may operate to cause complete clearing of the ' hæmolytic ' zones around streptococcal colonies on blood-agar plates. Firstly, absorption of the blood pigment into the colonies ; the appearance of these certainly often suggests that this is taking place. Secondly, accumulation of blood-pigment at the periphery of the cleared zone. Rother (1925) maintains that this will tend to happen whenever the reaction passes towards pH 5 in the immediate vicinity of the colonies, an electric field being set up between the acid area and the surrounding agar at pH 7·4 to 7·8 in which field the hæmoglobin migrates to the periphery. Thirdly, destruction of the blood pigment. Rother (1925) brings evidence to show that Hbg is actually partially destroyed when present in a medium in which streptococcus is growing and Seitz (1922)

was satisfied that he could trace oxidation of Hbg through MHbg to acid hematin in the clear ' hæmolytic ' zones around streptococcal colonies in blood-agar media.

In many descriptions all non-hæmolytic streptococci are classified as *S. viridans ;* this is partly due to the defect in Schottmüller's original classification, which did not make clear the existence of strains which were neither hæmolytic nor produced green-coloured colonies on blood-agar. Brown (1919), who has devoted an extensive monograph to this subject, has made good this defect in Schottmüller's classification. He describes a α type which corresponds to *S. viridans*, a β type which corresponds to the actively hæmolytic *S. pyogenes*, and a γ type which gives neither hæmolysis nor green pigmentation. This differentiation had already been made by the tube method, Lyall (1914) describing three types, (1) hæmolytic, (2) methæmoglobin forming, (3) neither hæmolytic nor MHbg forming. Rother (1925[2]) has shown that, although all hæmolytic streptococci tend to produce MHbg, *viridans* strains are much more active in this respect.

In view of what has been said above about the inferiority of the blood-plate as a criterion of hæmolytic activity there is much doubt about the value of refinements of interpretation of the appearances on it, such as Bryant's (1925) demonstration of a ' δ ' type producing no change on the surface but a green coloration in the depths of fresh blood-agar plates. The most common fæcal streptococcus comes under the ' γ ' class as regards hæmolysis, and the pneumococcus gives late hæmolysis of the ' α ' type. This is of special interest since Cole (1914) and Neill (1926) have brought forward evidence to show that the pneumococcus can be made to yield a hæmotoxin by methods suitable for liberation of endotoxin, and that this substance is a true antigen. It would therefore appear possible that there are two types of streptococcal hæmolysin, one secreted readily, non-antigenic and characteristic of the ' true ' hæmolytic streptococci, and the other not readily secreted, antigenic and specially associated with the *viridans* strains.

The tendency to formation of narrow ' hæmolytic ' rings around *viridans* colonies, especially if the cultures are kept alternately at 37° C. and in the cold, is emphasized by Brown ; and it is pointed out that possible confusion of these colonies with true β hæmolytic types is excluded owing to the fact that in the α type the red blood-cells underlying the colony remain intact. He proceeds to a further differentiation of α prime colonies, i.e. ones in which a narrow ' hæmolytic ' ring, absence of destruction of red blood corpuscles underlying the colony and absence of green colour are the characteristics. The absence of hæmolysis of blood corpuscles underlying the colonies of *S. viridans* is also noted by Holman (1916) and Lehmann (1926), and the latter describes a streptococcus giving this effect but no green as *S. lentus*.

A good deal of light has been shed on this subject of the appearances of streptococcal cultures on blood media by the recent work on bacterial

oxidations and reductions. McLeod and Gordon (1922) showed that a limited group of bacteria, notably the pneumococci and the so-called *viridans* streptococci, produced H_2O_2 if grown in culture media freely exposed to air and not too rich in catalase. Further, they found that the yellow-green colour characteristic of growths of such bacteria on heated blood media was proportional to the amount of H_2O_2 which they were capable of producing, and could as a matter of fact be simulated by the application of H_2O_2 to the medium. It was shown further by Avery and Neill (1924) and Neill and Avery (1924) that the reducing system set up in pneumococcal cultures was capable of oxidizing hæmoglobin to MHbg and lower products even in the presence of catalase, an effect which could not be obtained with solutions of H_2O_2 similar in concentration to those which occur in pneumococcal cultures. Lastly, Neill and Mallory (1926) have shown that streptococcal hæmolysin loses its activity on oxidation, but regains it on exposure to suitable reducing agents.

Hagan (1925) has applied some of the above observations to the explanation of the appearances seen in blood-agar plates. He has shown that in blood-agar containing fresh blood neither peroxide alone nor acid alone produce the characteristic colour of a *viridans* colony, but that if 2 per cent. lactic acid and a very dilute solution of H_2O_2 are applied to a hole in a plate, exactly the bottle-green colour of such colonies is obtained. Further, he shows that by growing *viridans* colonies at a low temperature in the absence of air they may be caused to produce in a typical way the picture of a β hæmolytic colony. Rother (1925[2&3]) has shown that if the normal sugar-content of blood-agar, which he estimates at 0·02 per cent., is raised by addition of glucose to 0·08 per cent., strains which would have been classed as hæmolytic on the ordinary blood-agar produce green colonies. From all this it would follow that the appearances of streptococcal colonies on blood media depend on the interplay of a very complex group of bacterial activities, amongst which hæmolysin production, acid production, reducing activities, peroxide production and possibly tryptic and peptic digestion of the blood corpuscles are the most important, and that the appearances can be modified at will by choosing conditions which will favour more the development of one or other of these activities, and hence the term *viridans* has little significance unless the conditions of growth are defined. As a matter of fact, the great majority of streptococci from all sources are peroxide formers in greater or less degree and can be shown by a sensitive medium such as heated blood-agar to produce the *viridans* effect.

It is open to question whether the green colours developed on heated and unheated blood-agar plates respectively are due to the same substance and the suggestion that the green colour developed in the latter is due to methæmoglobin (Grüter, 1909) which has a good deal of *a priori* improbability, certainly cannot be accepted in the case of the heated blood-agar plate in which there is no unaltered hæmoglobin present at the outset. The greater ease with which the colour is produced in heated blood-agar

plates, and the favouring effect of acid on its appearance in unheated blood make it probable that the colour is due to an oxidation product of hæmatin, the latter being first formed by the action of heat or acid on hæmoglobin or methæmoglobin. Exact knowledge on the subject is lacking, however.

Mutations.

It follows from Hagan's work and the deductions drawn from it above that much of the work on mutations from hæmolytic to green streptococci and conversely, such as those of Zoeppritz (1909), Hoessli (1910), Schnitzer and Munter (1921), Philipp (1924), and others, in which the observations are made on blood-agar plates, loses its significance. The subject is considered in more detail in the section on the streptococci of the mouth (p. 99).

Relations of Streptococci to other Bacteria.

As the question of the relation of the streptococcus to the pneumococcus will be discussed below it need not be considered here. There are two other relationships which may be discussed. Mellon (1917) draws attention to the reversion of a barred form of the diphtheria bacillus to a streptococcal form, and he discusses in a general way the relationship which he supposes exists between the non-hæmolytic streptococcus and the diphtheroid bacteria. Parish (1927) has also noted the occurrence of streptococcal forms in certain cultures of the diphtheria bacillus, but he could get no evidence whatsoever that the change indicated anything else than a phase of morphological differentiation. In any case, there are wide differences between the diphtheria bacillus and the streptococci on biochemical grounds, e.g. the streptococcus always tends to turn its cultures acid, whereas *C. diphtheriæ* tends to form alkali (Sierakowski, 1924; Abt and Loiseau, 1925; Phelon, Duthie and McLeod, 1926). Further, no streptococcus produces catalase, whereas most or all diphtheroids do (McLeod and Gordon, 1922); also it was noted by Bocchia (1909) that *C. diphtheriæ* was particularly sensitive to pyocyanase and streptococcus quite indifferent.

The other relationship to be considered is that of *S. pyogenes* of man to the streptococci pathogenic in animals. It is probable that most animals are susceptible to spontaneous streptococcal infections, and this will be discussed later; there are, however, two streptococcal diseases of larger animals with which man is much in contact, and in regard to which the identity of the streptococcus with those which invade the human body is important: these are bovine mastitis and strangles in the horse. The former is the more important, since the abundant opportunities of infection through milk are obvious. Sherman and Albus (1918) point out that there are two types of streptococci commonly present in milk, (1) a form derived from mastitis and related to *S. pyogenes*, (2) the true lactic acid streptococci. These they find distinct in several respects, e.g. the *pyogenes* variety is a less active

lactic acid producer, it has a less active reducing mechanism, it clots milk more slowly and it is also distinct in not growing below 10° C.

Brown (1920) has pointed out that when grown in 1 per cent. glucose broth, the human and bovine hæmolytic strains are distinguished by the degree of acidity they produce. The bovine form not ceasing to grow till a reaction pH 4·4 to 4·6 is reached, whereas the human strains stop growing at a reaction of pH 5·1 to 5·4. Ayers and Rupp (1922) concur in regard to this point of difference between bovine and human streptococci of *pyogenes* type, and add that these types are also differentiated with great regularity by the ability to split sodium hippurate possessed only by the bovine strains. Smith (1929), however, on re-investigating the matter did not find either the tests of Brown or Ayers and Rupp adequate for differentiating between bovine and human strains.

In regard to the equine strains, the differentiation appears less easy. The organism responsible for this disease and described as *Streptococcus equi* in Holman's classification is not infrequently met in human beings, and Lignières (1910) resorts in regard to it to the test which has been the ultimate criterion in the pasteurella group, i.e. relative pathogenicity to different animals. This character, he points out, is a constant one, and must be distinguished from virulence, which is a variable one.

Suggested Limited Scheme of Classification.

Before proceeding to describe the streptococci in detail some sort of classification is essential, for they are sufficiently diverse to make descriptions of certain types quite inapplicable to others. It seems to the writer that Andrewes' (1913) attitude towards this question is a sound one. That is to say, that both a rigid streptococcal classification into well-defined types and the conception that all streptococci belong to a single type subject to variations under conditions of growth, &c., are unsound, and that the streptococci are rather a group of bacteria with a small number of well-defined types adapted to different conditions of life, and many intermediate forms linking one type with another.

Departing from this conception and taking into account the work already surveyed, the classification most justified seems to be a primary division into two main groups: hæmolytic and non-hæmolytic. The effect of a small quantity (0·5 to 0·1 c.cm.) of a culture in 20 per cent. serum broth for 15 hours at 37° C. on 0·5 c.cm. of 5 per cent. suspension of washed ox blood when they are incubated together for 1½ hours is taken as the criterion of hæmylosis, i.e. the capacity to secrete a filterable hæmolysin. No further subdivision of the hæmolytic group seems to be justified at the present time, since even all the intensive work of the last few years has failed to reveal any certain criterion by which a given strain of hæmolytic streptococcus may be related to erysipelas, scarlet fever or any other disease (Stevens and Dochez, 1926). The non-hæmolytic group, however, contains types of streptococcus sufficiently distinct to make further subdivisions of the group necessary.

The subdivisions suggested are :

(1) Facultatively aerobic or anaerobic.
(2) Strictly anaerobic.

(*a*) The mouth streptococci.
(*b*) The bowel streptococci.
The anaerobic streptococci.

Of these subgroups 1 (*a*) probably contains the greatest diversity of organisms ; this, as has been pointed out above, is shown by their behaviour as antigens, and further evidence for it is the extraordinary variety in the morphology of their colonies when grown on a special medium containing a high percentage of heated blood (Warren Crowe, 1921, 1923, 1924). Taking it then for granted that groups of some complexity are being described rather than rigidly fixed types, the chief characteristics differentiating the above divisions and subdivisions of the streptococcal group are the following :

1. *Hæmolytic streptococci* are usually observed as chains of moderate length of well-rounded cocci, and grow readily on agar and broth, yielding cultures of good viability. They have for the most part active pathogenicity for laboratory animals, and are usually fermenters of salicin.

2. *The non-hæmolytic streptococci.* (1) Facultatively aerobic or anaerobic streptococci. (*a*) *The mouth streptococci* show as a rule long chains of organisms which are often elongated rather than round. They grow badly in the ordinary culture media and tend to die off rather rapidly. They are only slightly pathogenic to animals. They tend to produce a good deal of H_2O_2, and many of them are characterized by fermentation of raffinose ; but the group contains forms diverging widely from this description. (*b*) *The bowel streptococci* are plump, slightly oval, and almost invariably appear in short chains, or as diplococci. They are relatively resistant to drying, heat and antiseptics ; are active producers of H_2S, and have little or no virulence for laboratory animals. Their characteristic fermentations are mannitol and æsculin. (2) *The anaerobic streptococci* are at once distinguished by their inability to live in the presence of traces of oxygen : they are most often non-pathogenic for animals.

Streptococci Readily Secreting a Filterable Hæmolysin.

By J. W. McLeod.

Morphology and Staining.

The streptococcus is by definition a spherical organism with a tendency to grow in chains. This description fits most closely the hæmolytic and actively pathogenic forms. The individual cocci are usually about 1μ in diameter, and as in other small coccal organisms there is no detail of structure. All morphological variations depend, therefore, on variations in the length of the chain and irregularities in the shape and size of the individual cocci.

Length of chain varies a good deal under the conditions of growth. In the sero-purulent exudate of a commencing superficial lesion of the

finger, it is not uncommon to find a hæmolytic streptococcus growing almost entirely as coccal and diplococcal forms, with perhaps a few chains of 4 or 6 elements. In definitely formed abscesses, however, chains of considerable lengths, 10 elements and upwards, are the rule. In colonies on solid media again, there may be very little in the arrangement of the cocci one to another to suggest that the organism is a streptococcus, the appearances in smear preparations being more suggestive of a staphylococcus, but a minority of streptococci form well-defined chains even when growing on solid media. The medium, however, which can be most relied on to reveal the typical chain arrangement of the cocci is a fluid one—broth or serum broth. So much is this the case that the microscopic and macroscopic appearances of cultures of streptococci in broth have formed the basis for two of the older classifications of streptococci which were published about the same time, namely, those of Lingelsheim (1891) and Kurth (1891). Holman (1916) and Kinsella and Swift (1918) maintain that it is characteristic of hæmolytic and pyogenic streptococci to have the elements in the chain compressed so as to be longer in the axis at right angles to the chain, whereas non-hæmolytic streptococci tend to diplococcal arrangement within the chain and to elongation of the elements in the direction of the axis of the chain. The writer's observations are similar. Several unusual morphological variations may be met, such are the appearance of giant forms, i.e. 3 or 4 times the normal diameter. Taddei (1909) found that these were specially likely to appear in broth cultures containing caffein. Another is the appearance of bacillary forms from excessive elongation of the elements or defective division. Norton, Rogers and Georgieff (1921) maintained that such forms could be obtained with any strain if it was subcultured long enough on blood-agar at 10-day intervals. Kermorgant (1922) found that if the blood of an animal which was beginning to develop streptococcal septicæmia was drawn, defibrinated and incubated, then bacillary forms were particularly prone to appear during the first 12 hours of incubation, although nothing but streptococci could be recovered from it in subculture.

In some of the older papers on the streptococci, reference is made to arthrospores. These were unusually large and deeply stained cocci, occurring at intervals through a chain, and were thought to be associated with branching of a streptococcal chain. They had none of the peculiar powers of resistance to heat, &c., characteristic of bacillary spores (Vincent, 1902 ; Hillier, 1906). Capsule formation as it is seen in preparations of the pneumococcus or '*S. mucosus*' does not occur. Bail and Kleinhans (1912), however, maintain that the existence of a capsule around streptococci may be observed rather irregularly in the animal body, but becomes visible *in vitro* if the organisms are exposed simultaneously to the action of leucocytes and serum. Bordet also refers to capsule formation.

The hæmolytic streptococcus stains well with all the usual basic aniline dyes, and it is particularly well defined by dilute carbol fuchsine. It is

frankly Gram-positive, but not so markedly so as the staphylococcus, and may often take the stain irregularly or rather lightly, particularly in preparations from old cultures. Jaffé (1912) describes two strains which were invariably Gram-negative on subculture. This must vary with the character of the medium and the particular modification of Gram's staining method which is used. Kinsella (1918) notes a tendency to loss of Gram-positive staining with increase of virulence.

Cultivation.

The streptococcus occupies a position amongst pathogenic bacteria intermediate between the strictly parasitic forms, such as the gonococcus and *B. pertussis*, which are difficult to cultivate, and the enteric group of bacteria, which grow with the greatest freedom on artificial media even of the simplest constitution. There seems to be complete unanimity amongst all authors who have investigated the point, in the conclusion that the streptococcus will not grow in synthetic media prepared entirely from substances of known constitution, even when these include sugars and a variety of amino-acids (Koser and Rettger, 1919; Braun and Cahn-Bronner, 1921; Krasnow, Rivkin and Rosenberg, 1925).

Orla-Jensen (1919), who includes the streptococcus amongst his group of true lactic acid bacteria, maintains that it is characteristic of this group that they do not utilize the nitrogen of free NH_4 or amino-acid, but prefer for some obscure reason, to strip their nitrogen from more complex protein molecules. This observation fits well with those of Wyon and McLeod (1923) and Gordon and McLeod (1926) who found that *S. pyogenes* was amongst the bacteria of which the growth was inhibited by high concentrations of tryptic digests, the amino-acids mainly responsible for the inhibitory effect being apparently, glycine, cystine, tryptophan and phenylalanine. Whitehead (1924) observing the effects of various fractions of a tryptic digest on streptococcal growths concluded that tryptophan was not needed by this micro-organism, but that the peptides and diamino-acids contained in the precipitate obtained with phosphotungstic acid were required. A number of independent investigators therefore indicate that the nitrogenous nutrition of the streptococcus can only be maintained by protein of some complexity such as occurs in various peptone preparations. The older work of Bainbridge (1911) had made it clear that the higher protein molecules were unsuitable for bacterial nutrition, and therefore it is probable that it is the intermediate products in protein disintegration, the polypeptides, on which the streptococcus depends.

The importance of the non-nitrogenous organic constituents of a medium for promoting the growth of the streptococcus have been less investigated. As is the case with other bacteria, growth is markedly promoted by the presence of a fermentable sugar, and Kendall, Day, Walker and Ryan (1919) suggested that sugar may be used for building nucleic acid as well as in furnishing energy by its combustion.

Data with regard to the value of the salts of the fatty acids are scanty. Brown (1921) pointed out that the introduction of 1 per cent. of sodium citrate into a medium helped to differentiate between *S. pyogenes*, on which it exerts an inhibitory effect, and *S. fæcalis*, the growth of which is promoted. Neither is there much information about the effect of fats. As early as 1889, Fraenkel had observed that growth was increased by adding 5 per cent. glycerin to agar. In 1905, he noted that by incorporating fat in a medium, the growth of '*S. mucosus*' is much increased, but he does not mention any favouring effect on the growth of *S. pyogenes* obtained in the same way, although it is not clear from his account that this point was directly investigated. Ayers and Mudge (1922) have, however, examined the subject more thoroughly, and they find that where a medium prepared from peptone and autolysed yeast extract is used, the addition of even minute amounts of oils or fats greatly stimulates growth. Since the result is just as marked with mineral oils as with butter fat, it is quite likely that it is the physical effect of incorporating fat in a fluid medium rather than the provision of a substance which the bacteria utilize as a foodstuff which is important. It is possible that the minute amount of fat finely distributed through meat extract may be an important element in the growth-promoting effect of that preparation, but this point is not discussed in the paper of Ayers and Mudge.

The investigation of the value of inorganic salts in the case of bacteria which will not grow in simple synthetic media is difficult, since they are contained in variable amounts in substances like peptone ; Whitehead (1923), however, has shown that the NaCl content of a medium may be reduced to 0·1 per cent. without diminution of growth, and Meader and Robinson (1920) have found that the phosphate content of a medium may be much reduced without rendering it incapable of growing the streptococcus ; Whitehead's (1926) experiments, however, convinced him that the presence of inorganic phosphate was essential for rapid growth of streptococcus in tryptic casein broth.

Turning from the consideration of the role of the different constituents of a medium to the nature of the media which give the most profuse growths of the streptococcus, we find that the peptone salt medium which may suffice to give growth is greatly reinforced by addition of meat extract, and still further improved by addition of fresh serum, blood or fluid exudate, such as ascitic fluid. The nature of the action of these substances is obscure. Kligler (1919) has supposed that vitamin content is concerned, but McLeod and Wyon (1921), Wyon and McLeod (1923) and Wyon (1923) could find no evidence that these effects could be attributed to any of the known vitamins important in animal nutrition. In this opinion they were supported by the work of Ayers and Mudge (1922). It is probable that these substances influence growth by a physical effect such as Hitchens (1921) has found to be exercised by small percentages of agar incorporated in fluid media, and Platt (1927) has observed from addition of gelatin, and that at the same time they present to the bacteria

a complex mixture suitable for their nutrition. Both Mueller (1922) and Freedman and Funk (1922) have shown that the elements which promote streptococcal growth may be removed from meat extracts by boiling with charcoal. The difficulty in identifying such substances is emphasized by Mueller's very elaborate but unsuccessful efforts to do so.

Although the streptococcus will grow in undiluted fresh serum, being a serophyte in the sense of Wright (1915), it does not grow by any means so freely as the pneumococcus, and there are marked variations in the capacity of different strains to grow in serum (McLeod, 1921). Optimal growth is obtained in broth containing 20 to 50 per cent. of serum.

Characters of the Growth in Various Media.

In broth or serum broth, the appearances vary from general turbidity to granular or flocculent deposit with clear supernatant fluid. A combination of the two appearances is the rule in 24-hour cultures. In litmus milk acidification with slow and partial clotting or absence of clotting occurs (Andrewes and Horder, 1906; Gorini, 1926). Gorini, however, points out that it is only necessary to add peptone, Liebig's meat extract or yeast extract to get rapid clotting of milk by *S. pyogenes*. The rapid and complete reduction of the dye characteristic of *Streptococcus acidi lactici* Heim (1924) is not observed.

In bile or even glucose peptone water containing a considerable percentage of bile, there is no growth (Weissenbach, 1918); and unpublished work of B. Wheatley confirm this in so far as agar containing 40 per cent. of bile will grow *S. fæcalis*, but not true hæmolytic strains.

On potato the growth is very poor (Lingelsheim, 1891). On the usual nutrient agar surface growth is distinct, but not copious. The colonies when numerous tend to be fine. Distinctive points are their tendency to remain discrete, the growth of each colony being apparently limited by the substances produced by the neighbouring ones, and the cessation of growth after 24 hours. When numerous and fine, the colonies are round, very slightly raised, and relatively opaque and greyish white. Deep colonies are smooth, opaque and slightly yellow in colour, and vary in outline, being most often lenticular. Under a variety of circumstances much larger colonies may develop; this is particularly the case when only a few develop and in primary cultures: in fact, the large size of colony which may appear in the depths of an agar tube to which several cubic centimetres of fluid from an infected joint have been added is surprising. The addition of various enriching fluids to the medium has a somewhat similar effect, and it is observed in the larger colonies that a good deal of diversity of shape may occur. We are not at present in a position to say that any relationship between the morphological types of the colonies and other characteristics of the strain have been clearly established. It has, however, been suggested by Eagles (1924) that two distinct morphological types of colony exist, (*a*) a small greyish pin-point colony, and (*b*) a flat scale-like colony. Cowan (1922), however, had

maintained that by repeated plating and selection of colonies, it was possible to split several strains of streptococcus into rough and smooth variants, the one with a translucent bluish flat colony, and the other with a more opaque and roughly granular colony. The first she found gave a diffuse turbidity in broth, and the second a granular deposit. This work of Cowan makes it appear doubtful whether differentiations amongst streptococci based on the appearance of their colonies will prove useful, but Griffith (1927) has brought forward observations which are not easily reconciled with Cowan's. He found three morphological types of colony : (*a*) an opaque, flat, rough and coherent colony ; (*b*) a colony with raised opaque centre and thinner translucent margin which was never coherent ; (*c*) a large more translucent mucoid colony. His rough colony on the solid medium gave the uniform turbidity in broth characteristic of Cowan's smooth variant. Griffith claims a certain correspondence between morphological colony type and antigenic classification of his strains. An interesting observation bearing on this subject is that of Walker (1923), who found one or two colonies of a mucoid appearance cropping up in a culture taken from the pleura of an animal to which a hæmolytic streptococcus had been injected. The strain grown from these colonies much exceeded the strains isolated from other colonies in the plate, both in virulence and in hæmolytic activity. After repeated subculture it lost its peculiarities, and numerous attempts to secure a similar variant by repeated passages of the original strain through the rabbit all failed. Walker considered that these observations had a bearing on the occasional occurrence of serious epidemics of streptococcal sore throat, which might depend on the sudden emergence of variants of high virulence like that described above. If it does prove possible to establish a relationship between the varied morphological appearances of colonies, as for example, they appear on Crowe's medium, and their pathogenic significance, much time and effort may be saved.

Biochemical Reactions.

Saccharolytic and Amylolytic Activities.

The fermentative action of the streptococcus has been more extensively investigated than any other of its biochemical activities, and a good deal has already been said above that bears on this subject and need not be repeated.

It is claimed in the work of Kendall and Farmer (1912), that the streptococcus is essentially an acid-forming bacterium, and that it is one of the few bacteria that produce acid even in sugar-free media, apparently splitting it off from the carbohydrate radicles present in the proteins. They found that in media containing 1 per cent. of fermentable sugar as much as 75 per cent. of the sugar present might be converted to acid by some strains. Kendall, Day, Walker and Ryan (1919) investigating a very large collection of strains found that d. glucose, d. mannose, fructose, d. galactose, maltose, lactose, saccharose, trehalose and salicin were always

fermented, that the product was invariably acid and that CO_2 was often evolved, whereas dulcitol amongst the hexoses, and many pentoses and heptoses were not attacked by any streptococcus. Maass (1913) gives very similar lists of substances fermentable and non-fermentable by the streptococcus.

Ayers, Rupp and Mudge (1921) found that two hæmolytic streptococci of human origin both produced NH_3 and CO_2 when acting on peptone but not on dextrose, and they point out that the ordinary methods are useless for detecting CO_2 production, and that an Eldredge and Roberts (1914) tube must be used. Jones (1920) found that streptococci produced more acid when serum was added but less if the percentage of peptone in the medium was augmented.

Stevens and West (1922) investigating the fermentative action of extracts of ground streptococci found invertase present, but no ferment capable of disintegrating starch or dextrose.

Rosenthal and Patai (1914[1]) had demonstrated amylolytic action with filtrates of a streptococcal strain, probably not *S. pyogenes*, and Tongs (1919) using a plate in which starch was incorporated and also asparagin as coferment, got evidence of amylolytic action, particularly with the hæmolytic streptococcus, but not with all strains. There are not many records of investigations with regard to the product of fermentation when the streptococci acts on sugars, but they agree that lactic acid is the chief product. Sherman and Albus (1918) distinguish between pyogenic streptococci of bovine origin and milk-souring streptococci by the amount of lactic acid which they produce, the latter producing more than 0·75 per cent. and the former less. A similar distinction is made between hæmolytic and non-hæmolytic streptococci by Langwill (1924) who found that the ratio of volatile to non-volatile fatty acids formed in the course of fermentation was 1 to 7·7 for the latter, and 1 to 5 for the former. The figure obtained for lactic acid corresponded very nearly to that got by subtracting volatile from total acid. The nature of the volatile acids formed was not very thoroughly investigated, but a certain amount of evidence for the presence of acetic and propionic acids was obtained.

The fermentative action of the hæmolytic streptococcus may be summed up as follows : It belongs to a group of organisms which attack a wide variety of sugars and ferment them with great readiness, the chief product of fermentation is lactic acid, although some volatile fatty acid is also formed. No formation of CO_2 is detected in the ordinary Durham fermentation tubes, although it is claimed that its production can be shown by special methods of observation. Hæmolytic streptococci are also capable of liberating acid in media which contain little or no free sugar. They are amongst those members of the group to which they belong, which ferment least actively.

Proteolytic Action.

Gross evidences of proteolytic action such as may readily be obtained with the staphylococcus or *B. anthracis* are not observed in cultures of

hæmolytic streptococci. With finer methods of observation, and with extended periods of culture, however, evidence can be obtained that they can disintegrate certain proteins at all events. Thus Stevens and West (1922) were able to extract from streptococci a peptolytic ferment which acted on peptone, casein and serum with increase of the non-protein nitrogen present in solution. Its activity was trifling on serum, much greater on casein, and greatest on peptone. Kendall and Farmer (1912) found that small amounts of NH_3 were produced in cultures of streptococci, although much less than in those of most other bacteria. The NH_3 production, however, became extremely slight when fermentable sugar was added to the medium. Ayers, Rupp and Mudge (1921) also detected the production of NH_3 from peptone in cultures of hæmolytic streptococci.

Foster (1921) claims that a passage strain is distinguished from the stock strain from which it originated by more active production of NH_3 in the first six hours of culture and greater total production. Rosenthal and Patai (1914) maintain that streptococci produce more amino nitrogen in first 24 hours of culture than does *B. coli*, and that the highly pathogenic strains are more active in this respect. Apart from evidence of proteolytic action which can be obtained by chemical investigation, however, there is also some evidence of a grosser kind. Thus, Gorini (1926) maintains that pathogenic streptococci tend to digest the casein of milk slowly in acid solution, and Tissier and de Coulon (1920) and Tissier and de Trevise (1920), state that when maintained alive long enough in an acid medium, streptococci digest both casein and gelatin—the digestion of gelatin by certain types of fæcal streptococci, the so-called *S. zymogenes*, has, of course, long been recognized, but is not under discussion here. In the experience of the authors quoted, only hæmolytic and pathogenic streptococci showed this proteolytic action. Tongs (1919), has demonstrated the digestive powers of the streptococci for casein in a convincing way, by incorporating milk in agar plates and obtaining areas of clearing around streptococcal colonies. Meader and Robinson (1920) state that hæmotoxin from streptococcal cultures can destroy various glandular and muscular tissues as well as red blood corpuscles. Lastly, Frobisher (1926), describes a slow and progressive digestion of cooked meat in the cooked meat medium commonly used for culture of anaerobes. This is associated with a marked increase of the titrable amino nitrogen present in the culture, and it is due to a filterable emzyme to which he proposes to give the name histase. The late Professor Adrian Stokes drew my attention to this phenomenon, while I was working in his mobile laboratory in France in 1918, but I am not aware that he published an account of it. Frobisher states that this tissue-digesting power is distinct from properties of liquefaction of casein or gelatin or from hæmolytic activity.

The production of sulphide in media of which the only sulphur content is that combined in the protein molecule, is probably good evidence of proteolytic action, but no observation of this kind has been recorded to my knowledge. Ayers and Johnson (1924) have shown that *S. pyogenes*

when growing in a medium of nutrient broth plus 0·1 per cent. lead acetate and 0·25 per cent. $Na_2S_2O_3$ or $(NH_4)_2SO_4$ shows slight evidence of sulphide production and B. Wheatley (unpublished) working with Bürger's (1914) cystine-lead-acetate medium also got evidence of production of traces of sulphide by hæmolytic streptococci, which were, however, the least active of all streptococci tested. It is not clear that such observations need mean more than a demonstration of reducing activity.

Lipolytic Activity.

On this subject there has been very little work. Bergel (1922) following up earlier work of Neuberg (1907), observed pitting of beeswax plates where they were inoculated with cultures of hæmolytic streptococci, provided that they were kept at 52° C. Other observations on this subject are those of Thompson and Meleney (1924) and Gordon (1927) who obtained rapid hydrolysis of ethyl butyrate when it was incubated with heavy suspensions of streptococci in neutral fluids.

Oxidizing and Reducing Activities, Relation to Oxygen.

The streptococcus has features in common with other members of Orla-Jensen's group of true lactic acid bacteria—it lacks catalase, may, and often does, produce H_2O_2, and is microaërophilic in Beijerinck's sense. Its inability to tolerate a great deal of oxygen is emphasized by the observation of Oliver and Perkins (1919) that most streptococci will grow better when the normal oxygen tension of the atmosphere has been partly reduced, and Karsner, Brittingham and Richardson's (1923) observation that the number of colonies developing from an inoculation of hæmolytic streptococci is put down by 50 per cent., if the oxygen content of the atmosphere is raised above 80 per cent.

Anaerobic conditions do not seriously impair its growth.

Observations on the reducing activities of the streptococcus are mostly 25 or more years old, but they are unanimous in the conclusion that the hæmolytic types have very slight reducing activity. This was the finding of Lingelsheim (1891), Kurth (1891) and Pasquale (1893) for sodium indigo sulphonate solutions incorporated in agar, of Gordon (1904) for neutral red and of Sherman and Albus (1918) for litmus, methylene blue and indigo carmine, while McLeod and Gordon (1925) found them to be amongst the bacteria devoid or almost devoid of power to reduce cystine and its compounds. To sum up, therefore, the hæmolytic streptococcus appears to be a bacterium of high fermentative activity; deriving most of the energy it requires from such processes and mainly indifferent to oxygen supply. Its reducing activities are slight, but it is capable of oxidations on account of its capacity to form H_2O_2, and it is possessed of real though not very obvious proteolytic powers which are probably closely associated with its pathogenic activities.

Chromogenic Activity.

Durand and Giraud (1923) noted the existence in a series of 125 strains of about 9 per cent. which produced yellow to ochre or even red colonies

in media containing 1 per cent. of starch and protected from free access of oxygen. This observation does not appear to have been made by any one else and H. Channon (unpublished) was unable to detect it amongst any of a smaller series of strains.

Biological Characteristics.

Pasquale (1893) found that the average viability of a streptococcal culture was 40 days on ordinary nutrient agar or broth, but that in dried preparations, especially blood, it might persist alive for months. Kurth (1891) found dried streptococcal preparations viable for 5 to 7 weeks, a period which, he points out, corresponds to that of the infectivity of the epidermal scales in scarlatina.

Meleney and Zung-Dau Zau (1924) found 0·2 per cent. sodium citrate plus 0·5 per cent. gelatin solution in water the best fluid for preserving streptococci. In this an inoculum of small numbers had not died off completely after 15 days at 20° C., and showed undiminished numbers after 72 hours at the same temperature.

Schwarz (1924) found 39 to 43° C. the highest temperature permitting growth in broth and 41 to 45° C. the highest temperature allowing growth in blood. Lingelsheim (1891) found 14 to 16° C. the lowest temperature permitting growth, and that a temperature of 55° C. for 20 minutes or of 60° C. for 10 minutes was sufficient to kill long-chained streptococci. Ayers, Johnson and Davis (1918) observed that the hæmolytic streptococci did not survive a temperature of 60° C. for 30 minutes, but Beckwith and Rose (1925) while getting the same result for a streptococcal strain recently passed through the animal body found that an old laboratory strain could resist 65° C. for 30 minutes but not 70° C. Simon (1904) stated that a temperature of 63 to 64° C. for one hour rarely failed to kill the sediment of broth cultures.

It would appear from these observations that hæmolytic streptococci are often killed by short exposure at 60° C., and rarely survive 30 minutes at 65° C., but that to ensure absolutely their complete destruction, 70° C. for half an hour is necessary. The exact temperature required is, however, probably controlled by the condition of the culture and the nature of the suspending fluid, since Thompson and Meleney (1924), suspending streptococci in a dilute solution of salt and gelatin, found them to be killed by heating at 55° C. for 10 minutes, i.e. as readily as their ferments were destroyed. Dible (1921) who used heat resistance largely for distinguishing fæcal from other streptococcal types, found a short heating at 60° C. sufficient to kill all but *S. fæcalis*.

Pathogenic Action in Animals.

Spontaneous Infections.

The streptococcus is undoubtedly responsible for much natural disease amongst a wide variety of animals. Conditions such as ' strangles ' in

horses, and mastitis in cows, have long been recognized and are discussed in treatises on veterinary pathology and bacteriology. Apart from such conditions, however, there are many records of streptococcal disease in animals which have interest on account of the confusion to which they may give rise in experimentation, because of the additional light which they throw on the pathogenic characteristics of the micro-organism, and in view of their possible importance in causing contact infection in man. The commonest types of spontaneous disease due to streptococcus observed in animals have been a septicæmia of obscure origin associated with marked splenic enlargement and often with evidence of hæmolysis in the general circulation, and hæmoglobin stained exudate in the serous sacs. Horne observed such disease amongst rabbits, and Kutschera (1908) observed an epidemic of this kind amongst mice, although in some of his animals there were abscesses in the spleen and association of staphylococcus in small numbers in his cultures.

Where a local lesion precedes the development of the septicæmia, it appears to be most often situated in the respiratory tract. Holman (1914) thrice isolated the streptococcus from the lungs of guinea-pigs dying spontaneously with a pneumonic lesion. Blake and Cecil (1920) state that spontaneous pneumonia in the monkey, although usually due to the pneumococcus, is occasionally due to a streptococcus. Mathers (1918) described an epidemic amongst horses of which the chief features were high fever, mucous nasal discharge which invariably contained hæmolytic streptococci and in the severe cases, lobular pneumonia, empyema, and general hyperplasia of the lymphatic tissues. Bayne-Jones (1922) describes a somewhat similar condition, usually fatal, amongst cats, in which mucopurulent nasal discharge was followed by septicæmia with great regularity. Sherman (1919) found that one dog in three amongst 32 examined harboured hæmolytic streptococci in its throat. Most of these strains were found to be pathogenic to mice. Apart from these lesions of the respiratory tract the hæmolytic streptococcus is noted by Holman (1916) as being frequently associated with endometritis in guinea-pigs.

Infections due to Inoculation.

Effects of inoculation vary to some extent with the method of introducing the bacteria, with the susceptibility of the animal and with the degree of virulence of the strain, age of culture, &c. There is some difference of opinion as to which animal is most susceptible. For Fraenkel (1884) working with puerperal strains, and for Rosenbach (1884) using his *pyogenes* strains, mice appeared to be more susceptible than rabbits, but Pasquale (1893) considered that if relative sizes of animals were sufficiently considered, the rabbit was the animal most readily attacked. Schenck (1897) maintains that amongst rabbits, white varieties and English breeds offer least resistance. Cafeiro (1914) gives

the following data with regard to the relative susceptibility of some creatures to ascitic broth culture of a streptococcal strain.

Animal.	*Fatal Dose.*	
	Subcutaneous.	*Intraperitoneal.*
Dog	12 c.cm.	12 c.cm.
Guinea-pig	No effect.	3·5 c.cm.
Rabbit	0·01 c.cm.	0·001 c.cm.
Pigeon	No effect.	No effect.

The results of other workers are in general agreement with these findings as regards the relative susceptibility of various creatures (Lingelsheim, 1891 ; Kutschera, 1908). The more common methods of inoculation are subcutaneous, intravenous and intraperitoneal. These and almost any other method of inoculation with a strain of sufficient virulence give rise to a general septicæmia which runs its course in 16 hours to 4 days, and is distinguished by evidence of active hæmolysis within the body of the experimental animal in those cases in which the streptococci multiply most rapidly in the blood.

Besredka (1901), Breton (1903), Tchitchikine (1906), McLeod (1915), note such effects in rabbits, and Kuczynski and Wolff (1920) occasionally observed them in mice. The evidence of hæmolysis may take the form of staining of the walls of the blood-vessels, hæmoglobinuria, hæmoglobin-stained exudates in the serous sacs, or even complete laking of the blood found in the heart. Additional features are splenic enlargement and swelling of lymphatic tissues. With strains of lesser virulence and strains administered in small doses a much less rapid septicæmia may develop, with a considerable tendency to localization in the tissues ; splenic enlargement may be very marked ; there may be areas of hepatization in the lungs and swollen lymphoid tissues ; sometimes there are purulent accumulations in the serous sacs ; and, particularly in cases of massive intravenous inoculation, there are lesions of the joints. Arthritic lesions have been noted in rabbits by Jackson (1913), Henrici (1916), and Cowan (1924), and by Koch (1912) in dogs; Schloss and Foster (1913) observed transient septicæmia and arthritis in monkeys injected with a strain of hæmolytic streptococci isolated from the tonsil of a patient with arthritis.

Reichstein (1914) found that in these less acute septicæmias the streptococci at first disappeared rapidly from the blood, reappeared irregularly, and tended to be absent completely for some time before death. Weil (1911) had formed similar conclusions. Radice (1923) records a septicæmia following inoculation in the rabbit in which streptococci were recovered in increasing numbers up to the seventeenth day, but ultimately disappeared entirely, the animal recovering. Von Bonnsdorff (1899) records the death of a rabbit several weeks after inoculation, the streptococci only being recovered from one lesion in the body—a necrotic and swollen lymphatic gland. With a lesser degree of virulence, the

injected streptococci only cause a local lesion such as a subcutaneous abscess ; it is interesting in this connection to note Wallgren's observation (1899) that on making an injection to the peritoneum the streptococci may be disposed of in the peritoneum without obvious lesion, but an abscess is formed in the needle track in the abdominal wall. Sternberg (1911) maintains that in the rabbit a streptococcal infection is more likely to cause a hypertrophy of the myeloid tissue than either a pneumococcal or staphylococcal one, and Tongs (1921) observed that an amphophile leucocytosis invariably followed the injection of hæmolytic streptococci. He states that in animals resisting the infection this leucocytosis persists, but that in animals which are going to die a leucopenia ensues, the healthy amphophiles are replaced by degenerate ones and by myelocytes, and degenerative changes are also observed in the bone-marrow.

Special methods of infection. Erysipelas has been produced by cutaneous inoculation of cultures of a suitable grade of pathogenicity by many independent observers, e.g. Fraenkel (1889), rabbits; Metschnikoff (1887), white rats ; Raskin (1889), Schenck (1897), Amoss and Birkhaug (1925), rabbits and dogs ; Birkhaug (1926), rabbit. In much of this work the erysipelatous lesion was produced by strains which were not derived from cases of erysipelas. Direct infection of the lungs has been attempted in three ways : (1) inhalation ; Branch and Stillman (1925) using this method succeeded in infecting 13 per cent. of 216 mice with lobular pneumonia, while 50 per cent. developed a fatal septicæmia, whereas they could not infect mice with pneumococci unless they were first doped with alcohol. (2) Direct injection to trachea with needle and syringe. This, in the experience of Gay and Rhodes (1921), was never successful. (3) Infection by insufflation to the bifurcation of the trachea by catheter. Silfvast employed this method in 1899 with positive results similar to those obtained by Gay and Rhodes (1921) on rabbits, Wollstein and Meltzer (1913) on dogs, and Blake and Cecil (1920) on monkeys. An interstitial and lobular pneumonia with a marked tendency to the development of empyema, and with considerable damage to the framework of the lung and subsequent repair, was the lesion produced.

Few observers appear to have investigated the effects of direct inoculation to the nervous system ; the old work of Homén and Laitinen (1899) was very thorough, however. They found that a direct injection to a nerve was more likely to prove lethal than a subcutaneous injection of a similar amount of culture. The cocci apparently spread up the nerve to the cord producing there something like an ascending myelitis. Ayers, Johnson and Davis (1918) noted three strains which caused paralysis without death in the injected rabbit. Mendel (1922) found the intracerebral route of inoculation the surest for producing infection, the streptococcus injected often producing septicæmia after a long latent period. Duval and Hibbard (1925–6) also mention paralysis as one of the results of injecting lysate of streptococci from scarlatina. Irons, Brown and Nadler (1916) isolated a streptococcus from the tear-sac of a man

suffering from irido-cyclitis which for some time maintained the property of producing the same condition when injected intravenously to a rabbit.

Tchitchikine (1906) infected many rabbits by administration of streptococcal culture to the stomach, but was led to conclude that his positive results were due to contamination of the pharynx and fauces, and not to introduction of bacteria to the stomach.

The work of Duval and Hibbard (1927) shows that the susceptibility of the kidney to streptococcal intoxication, which may be assumed from clinical experience, is capable of demonstration in the dog, since small injections of culture or larger injections of endotoxin produce a nephritis with glomerulitis which is frequently fatal.

The question of the way in which the animal body disposes of streptococci is raised by Mathes and Schultz (1925–6). They found that after a massive intravenous inoculation to the dog the blood at first yielded 10,000 colonies per c.cm., but became sterile in 6 to 10 hours, although examination of the viscera only revealed scanty cocci. The explanation they found in an excretion of 1,000 per c.cm. in the bile. Richet and St. Girons (1911), however, working with the rabbit, concluded that streptococci injected are eliminated within 30 minutes by appendix, duodenum or stomach and not by the bile. Von Bonnsdorff (1899) had concluded that streptococci injected intravenously were not excreted through the kidney unless septicæmia proceeding to a fatal termination developed, i.e. the kidney was injured before it would allow streptococci to pass.

Hopkins and Parker (1918), who have studied the fate of inoculated streptococci in the animal body more carefully than any of the above, used the cat, insusceptible, and the rabbit, susceptible. They observed that in both the streptococci were at first removed rapidly from the circulation, and that in the rabbit only they reappeared later. They found no evidence of excretion of the cocci by the bile, and they do not record observations on excretion by the intestine. They noted that the cocci were held up in the visceral capillaries and destroyed in the liver and lung, but not so readily in the other viscera. In the cat they were mainly held up in the lungs. It was apparently because they multiplied in the muscle or muscle capillaries in the rabbit, and were fed back into the general circulation after a time from there that the septicæmia reappeared and eventually exceeded the capacity of liver and lungs to cope with it.

Properties of the Streptococcus Responsible for its Invasive Powers.

The streptococcus stands out amongst the pyogenic bacteria on account of the rapidity with which it invades the tissues, so much so that a widely spreading lesion is looked upon as characteristic of this micro-organism. It is also with this bacterium that some of the most convincing demonstrations of accentuation of virulence by passage have been made. Marmorek (1902) maintained that by alternating culture in a mixture of

broth and ascitic fluid with passage through the rabbit it was possible to raise the virulence of any strain of streptococcus, and so greatly was the virulence raised for some strains that a dose computed to contain at most 1 or 2 cocci became lethal. Meyer (1902) found increase of virulence by passage following Marmorek's method easy with scarlatinal strains, but very difficult with streptococci from anginas associated with acute rheumatism. In the writer's experience (1915) accentuation of virulence by passage was only possible with some strains. Beckwith and Rose (1925) give exact observations on this subject ; of two cultures from the same strain, one was regularly subcultured on artificial media, while the other was passed through a rabbit's pleura at intervals of a month, over a period of five years. At the end of this period 300 cocci was a fatal dose of the passage strain (intrapleural), while 166,000,000 of the corresponding ' stock ' strain were required to kill.

The question then arises, ' to what is this high virulence or capacity for developing high virulence due ? ' Does the streptococcus produce a potent toxin ? As a matter of fact the bacteria which do produce the most potent toxins are not for the most part active invaders of the tissue, e.g. *C. diphtheriæ* and *B. tetani*. These produce soluble toxins acting most obviously on the nervous system. The type of toxin, however, which is likely to convey high invasive powers to the bacteria producing it is one which paralyses the phagocytic and defensive cells. This aspect of virulence has been recently discussed by Todd (1927[1, 2, & 3]), who points out that there is a close association between virulence in streptococcal strains and their power to multiply in defibrinated human blood. He found, however, that although he could show a slight toxic effect on the bactericidal power of blood when the relatively thermostable skin toxin described by the Dicks was added to it, yet he could not demonstrate any connection between the yield of skin toxin given by a strain and its virulence to mice or power to grow in defibrinated human blood. He reached the conclusion that ' virulence is not dependent on the production of soluble toxin '. This is quoted here because it is necessary to point out that such conclusions are not justified without consideration of the older observations on streptococcal toxin, all reference to which is omitted in Todd's work.

It has been shown by the work of Besredka (1914) using his technique for obtaining endotoxin and by Aronson (1912) and Brugnatelli (1913) using the technique of anaphylatoxin production—digestion of bacterial powder in guinea-pig's serum—that it is much more difficult to get toxic substances from streptococci than from most other bacteria. Stoddard and Woods (1916), however, describe the production of nephritis by the injection of streptococcal detritus prepared by MacFadyean's technique for bacterial disintegration. Apart from these results, and the various toxic effects obtained with filtrates from old cultures of streptococci, (Laitinen, 1896 ; Schenck, 1897 ; Homén and Laitinen, 1899 ; Baginsky and Sommerfeld, 1900 ; Simon, 1904), which, may correspond to those

got with skin toxins by Lash and Caplan (1925, 1926), by Paraf (1925) and by Parish and Okell (1927), there exists a class of soluble toxic substance or substances produced by streptococci which are in all probability quite distinct.

There is an old controversy about the relationship amongst streptococci of hæmolytic power to virulence. In this discussion opinion is pretty equally divided. A considerable part of the very abundant literature on this subject was reviewed by the writer in 1915, and the opinion expressed, that although the view that hæmolytic activity indicated virulence was unjustified, the opposite statement that there was no connection between hæmolytic activity and virulence was as unsatisfactory. It was true that not all hæmolytic streptococci were virulent, but it was also true that few virulent streptococci were not hæmolytic. The situation has been summed up in exactly the same way in the most recent paper in which the question has been carefully examined (Cesari, Cotoni and Lavalle, 1927).

On *a priori* grounds it would seem not unlikely that a hæmolytic toxin was also a general toxin, and there is considerable evidence to show that the hæmolysin if not actually a toxin is at all events closely associated with a toxic substance which resembles it closely in its properties, McLeod and McNee (1913) showed that when rabbits relatively devoid of natural antibody to streptolysin were chosen they could be killed by repeated doses of filtrates of streptococcal cultures, and that the more hæmolytic the filtrate the more actively they were toxic. Blood destruction was not sufficient to account for death, and the rabbits with more natural antistreptolysin withstood many doses of the filtrate, although they eventually developed a definite anæmia. These findings have been confirmed by Cesari, Cotoni and Lavalle (1927). Confirmation has also been obtained by H. Channon in recent work not yet published.

De Kruif and Ireland (1920) have shown that streptolysin is secreted only for a short period at the start of growth in the culture—eighth to tenth hour. It is obvious that this toxic substance has a much better claim to the description of soluble toxin in the sense of secreted poison than the filtrates of old and disintegrating cultures which are probably rather of the nature of endotoxins. The streptolysin is thermolabile above 55° C. (McLeod, 1912 ; Neill and Mallory, 1926), and no one has yet succeeded in demonstrating antibody formation to it ; in both of these respects it differs from the skin toxin of *S. scarlatinæ*. There is, however, a variable amount of natural antibody, or at all events neutralizing effect in different animal sera (McLeod, 1912), and Gordon (1927) has shown that bile-salts suspend the action of streptolysin.

Both Clark and Felton (1918) and Havens and Taylor (1921) have described highly toxic filtrates obtained from cultures of streptococci in special media. These toxins were thermolabile and susceptible to very rapid spontaneous deterioration just like streptolysin, but in the opinion of these workers they were distinct from hæmolysin.

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One other toxic effect of streptococcal cultures which has been investigated is the production of leucocidin, i.e. of substances having a special effect in disintegrating and paralysing leucocytes. McLeod (1915) pointed out that in cases where marked intravascular hæmolysis characterized a streptococcal septicæmia in the rabbit there was also very definite disintegration of the leucocytes of the granular series. Levaditi (1918) studied this phenomenon more closely, and came to the conclusion that leucotoxic effects were marked and constant with hæmolytic streptococci, but not strictly related to hæmolytic activities. He was not able to demonstrate leucocidal effects with filtrates, and considered the leucocidin as a secretion chiefly elaborated by the cocci within the leucocytes. Nakayama (1920), however, has made a more exact study of leucocidins, using the delicate method of observation introduced by Neisser and Wechsberg (1901) i.e. the determination of interference with the vital activity of leucocytes by their loss of the power of reducing methylene blue. He convinced himself of the existence of a filterable leucocidin which corresponded closely to hæmolysin in the time of its production in the course of culture, and also in its thermolability and sensitiveness to oxidation, but he thought it differed from the latter in its closer association with virulence. Channon and McLeod (1929) have obtained more direct evidence of the production of streptococcal leucocidin.

All this work may be summed up in the statement that the existence of thermolabile toxic products distinct from the type of toxin which reacts in skin tests has been demonstrated repeatedly in cultures of hæmolytic streptococci, and that the toxic substances have never been very clearly differentiated from the hæmolysin, but that an exact parallelism between virulence and hæmolytic activity in streptococcal strains does not exist, since many independent observers have found that hæmolysin production may continue active in a strain which has lost virulence. It was suggested by the writer (1915) that virulence might depend chiefly on two factors: (1) activity as toxin producer, and (2) capacity for growing and producing toxin in the unchanged blood-serum of the animal concerned. The power of multiplying in serum or blood is the quality which is lost most readily on subculture on artificial media, whilst the continued hæmolysin production indicates the strain as one potentially dangerous. Such a criterion of virulence was found accurate for animal experiment in a limited series of observations (McLeod, 1915), and it was possible to demonstrate some correspondence between the severity of the lesion caused by a streptococcus in man, and its virulence as judged by its capacity for growing and producing hæmolysin in fresh human serum (McLeod, 1921).

Immunity.

The wide variety of animals susceptible to streptococcal infection on the one hand, and on the other the fact that negative ' Dick ' reactions are increasingly rare, the younger and the better protected from infection

the individuals examined are (Kinloch, Smith and Taylor, 1927) indicate that natural immunity to streptococcal infection is uncommon. Certainly the data available with regard to it are scanty. Bumm (1925) found in test-tube experiments that the scale of resistance of different bloods to streptococcal growth was: dog, marked; man, moderate; rabbit and horse, slight. It is also suggested, from the frequency of relapse in streptococcal infections like erysipelas and from the clinical observation that streptococcal septicæmias may terminate fatally after persisting for weeks or months, that the animal body is not easily immunized against the streptococcus. On the question of the degree of immunity which may be acquired by the animal body when it is stimulated by injections of dead or living streptococci, or both, there is abundance of information. Before considering this, it is necessary to discuss the unity of hæmolytic streptococci from the standpoint of immunity, since what is said with regard to immunity might not apply equally to a variety of groups of hæmolytic streptococci, if such existed.

Attempts at Serological Classification of the Hæmolytic Streptococci.

The attempts to classify streptococci by methods depending on their antigenic characters, have been very numerous, and the results are rather bewildering. At one extreme, Smith (1927) defines ten separate groups by agglutinations, and describes certain associations between the morbid process from which streptococci have been obtained and the groups into which they fall. At the other extreme, Kinsella and Swift (1918), depending on reactions of complement fixation, find all hæmolytic streptococci examined identical. Valentine and Mishulow (1921) fail to make any clear classification of hæmolytic streptococci from the respiratory tract by agglutination methods; and in the Special Report, No. 57, of the Medical Research Council, 24 streptococci from war wounds were stated to be identical by agglutination methods. Lastly, Stevens and Dochez (1926), after extensive studies of agglutin absorption, conclude that the antigenic individuality of each strain is a more marked feature than any group characteristic, and that, for example, no clear distinction between certain streptococci of the erysipelas and scarlatinal groups can be made by such methods.

The great difficulty in assessing the value of this work is due to the fact that few observers have made an attempt to correlate their work with that of those who have preceded them. Dochez, Avery and Lancefield (1919) examining 100 strains of hæmolytic streptococci, mostly from the respiratory tract, by agglutination methods, determined the existence of four well-defined immunological groups, in which the majority of their strains could be placed. There was also found a residuum of unclassified strains. Where the strains were of sufficient virulence, it could be shown that animals were protected by the prophylactic injection of homologous serum, but not by that of serum for a streptococcus belonging to another group. Very similar results were obtained by

Havens (1919) and Hamilton and Havens (1919) about the same time. In the work of Tunnicliff (1920), Gordon (1921), Eagles (1924), Durand and Sedallian (1923, 1925), Birkhaug (1925), Amoss and Birkhaug (1925), Kinloch, Smith and Taylor (1927) and Griffith (1927), there is no mention of an attempt at correlation of their immunological groupings with those arrived at in the earlier work referred to.

The general impression derived from all this work, however, is that the most marked antigenic distinction is between the streptococci associated with scarlatina and all other hæmolytic streptococci, although the former are not a homogeneous group, and strains identical with some of them may be found in other conditions. Birkhaug (1925) and Amoss and Birkhaug (1925), claim that a majority of erysipelas strains form a single group marked off from most other streptococci by their antigenic individuality. Another method of grouping streptococci in accordance with their antigenic qualities is that adopted by McLachlan (1927). He determined the extent to which the skin toxins obtained from strains other than scarlet strains could be neutralized by antisera to skin toxin derived from scarlet strains. He found that there was no clear distinction between scarlet and non-scarlet strains to be made in this way. The chief distinction was that strains of streptococci from scarlet fever cases were more uniformly high skin toxin producers. Cumming (1927) has suggested that an element of confusion may have been introduced in this work owing to the classification of pseudo-hæmolytic streptococci—those which only produce clearing on human blood-agar plates—as true hæmolytic streptococci.

It would seem from all this that immunological methods as at present developed, do not permit of any rigid distinction between strains of streptococci associated with one type of disease, and those associated with others, and that there is either fluctuation of type under varying conditions of parasitism or a lack of any close association between type and pathogenic quality.

At all events, there is no reason at present for assuming that the experience acquired with regard to the development of immunity to one type of hæmolytic streptococci cannot be applied to all. It is, therefore, admissible to present and analyse the observations on this subject as a whole.

Acquired Immunity resulting from Introduction of Streptococci or Streptococcal Products.

General immunity. An important factor in all work of this kind, is the great variations in virulence to which the streptococcus is liable as the result of passage. Accentuation of virulence has already been described. The converse of this apparently may also happen ; a streptococcus which persists for a long time in the animal body without killing it, may exhibit an equally surprising loss of virulence. But in order to get experimental results which are reliable, it is necessary to work with

strains which kill the controls with great regularity, hence most of the work on this subject depends on results obtained with passage strains of high virulence, i.e. strains of which the cultures in such extremely small amounts as 0·001 to 0·0001 c.cm. represent 1,000 to 10,000 m.l.d. As a result, an immunity which stated in multiples of the m.l.d. resisted seems marked, may actually be an immunity to very small numbers of cocci.

The difficulties in developing a solid immunity to streptococcal infection were recognized from the outset of this kind of work. Thus, Friedrich (1895), injecting heat-killed broth cultures to man, found that, although he got diminished reactions on repeating his injections at short intervals, yet he obtained reactions equal to the first, if a larger interval was allowed to intervene. Lingelsheim (1891) failed to immunize mice with heat-killed cultures. Pasquale (1893) found that rabbits long immunized with dead and finally with relatively avirulent live cultures, could not withstand 0·1 c.cm. of virulent culture. Tchitchikine (1906) could not immunize rabbits with streptococcal culture administered to the stomach. Silberstrom (1906), like Pasquale, failed to produce a solid immunity in the rabbit. Killian (1924, 1925) produced a very definite immunity of short duration in mice by repeated intravenous or intraperitoneal injections of heat-killed cultures, but failed to produce any immunity by subcutaneous injections or by administration *per os;* further, the immunity obtained by the other methods was only exerted against the corresponding strain. Schloss and Foster (1913–14), however, found that a monkey injected repeatedly with a streptococcus which at first produced septicæmia and arthritis, ceased after three or four months to react at all. In the writer's own experience (1914), immunization of the rabbit against strains of high potential virulence, i.e. such as undergo rapid accentuation of virulence on passage, is difficult.

Local immunity. Gay and Morrison (1923[1]) suggest a scale of graded resistance exhibited by different tissues to streptococcal invasion. They state that the resistance of skin, muscle and connective tissue is good, that of the peritoneum fair, and that of the pleura and meninges poor; they found that an animal could survive a 1,000 times larger dose administered to the peritoneum than to the pleura. In a series of publications, Gay and Morrison (1923[2]), Gay and Linton (1925–6), Gay and Clark (1926) and Gay, Clark and Linton (1926), emphasize the importance of a non-specific local immunity by showing how considerably it is possible to raise the resistance of the pleura to streptococcal infection by injections of non-infective materials, such as aleuronate, gum, broth, &c. Goldschmidt and Schloss (1926), observed that the repeated opening of a dog's abdomen under aseptic conditions, with a view to collecting peritoneal fluid, &c., caused a non-specific increase in peritoneal resistance to infection.

Besredka and Urbain (1923) describe a method of obtaining local immunity by applying dressings of filtrates from streptococcal culture (the streptococcus is grown repeatedly in culture filtrates till they no

longer support growth) to the skin of a guinea-pig for 24 hours. No immunity can be obtained by intraperitoneal injection of the same filtrates, and Rivalier (1923), has emphasized the extremely local nature of this protection, since the streptococci, though failing to produce any local lesion, may pass on to cause a fatal septicæmia. Carrère (1925), however, using Besredka's ' bouillon-vaccine ', and instilling it repeatedly for 24 hours to one eye, and then infecting both with a similar instillation or injection of active streptococcus, found that the treated eye was very definitely protected. And Gloukhoff (1927) describes good results in the treatment of erysipelas by injection of such filtrates at the advancing margin of the lesion.

Mechanism of Immunity.

The factors which, in so far as we know them at the present time, are likely to play a part in immunity to the streptococcus, are, in the case of natural immunity, insusceptibility to streptococcal toxin, bactericidal action of serum and tissue fluids, and the phagocytic activities of leucocytes and fixed tissue cells. In acquired immunity, in addition to the above, various antibodies with agglutinating, antitoxic or bactericidal effect as well as opsonins or bacteriotropins may come into play, and there is further the influence of histological changes which may be obvious or imperceptible, in producing local immunity.

There is no doubt that till quite recently, and ever since Metschnikoff (1887) pointed out the active phagocytosis of streptococci by polymorphonuclear leucocytes and mononuclear cells in the periphery of erysipelatous areas, there has been a tendency to concentrate attention on phagocytosis, opsonins and bacteriotropins, and especially the polymorphonuclear leucocyte, as the important factors in antistreptococcal immunity.

If the very numerous observations on the bactericidal effect of various body fluids are considered, it is seen that apart from the leucocytes, little or no bactericidal effect occurs. But, Havens (1919), and Hamilton and Havens (1919) do describe some definite bactericidal effect which can be observed after 4 hours' contact between a blood broth culture of a strain and a mixture of the serum appropriate to that strain with fresh serum. They do not say whether the bactericidal effect persists, and Lehmann (1924), who pointed out that pneumococcus, staphylococcus, and hæmolytic streptococcus stand out from other bacteria on account of their marked resistance to the bactericidal action of serum, says further (1926) that hæmolytic streptococci, although invariably decreased in number during the first 12 hours of contact with serum, grow freely in it after that period. Ruediger (1906), Lomonaco (1910), Weil (1911), and Reichstein (1914), all refer to the absence of bactericidal action for streptococci in antistreptococcal serum, and even in the blood of immunized animals. The same idea is indicated in Wright's (1915) classification of the streptococcus amongst the serophytic bacteria. In the writer's experience (1915) also no significant bactericidal power has

been observed in human or animal sera. The variation in speed with which different streptococci grow in serum, however, has been found to be marked. Ruge (1923–4) and Philipp (1924) have actually proposed speed of growth of a streptococcus in the defibrinated blood of the patient who is infected by it as a criterion of its virulence, and although this is by no means an infallible test, it appears to have a rough accuracy, 70 per cent. to 80 per cent. (Gambetti, 1924; Schwarz, 1924). Ruge emphasizes the importance of the leucocytes in restraining the growth of the less virulent strain. The importance of the leucocytes in producing bactericidal effects *in vitro* had already been demonstrated by Wright and his collaborators (Wright, Colebrook and Storer, 1923; Colebrook, 1925, 1926).

A number of workers have attempted direct comparisons between the bactericidal effects of serum, of blood and of leucocytes prepared in various ways. Simon (1901) investigated the bactericidal effect of 'aleuronal' pleural exudates from rabbits, and found that the washed leucocytes from such exudates suspended in physiological salt solution were more actively bactericidal to streptococci than either whole exudate, or rabbit serum, but that against virulent strains this bactericidal effect was much diminished, and he proposed resistance to the bactericidal effect of leucocyte suspension as a test for virulence. Bail and Kleinhans (1912) pointed out that disintegration of leucocytes by freezing and thawing increased the bactericidal effects of serum leucocyte mixtures, except where the leucocytes were mixed with immune serum. The inference was drawn that in presence of immune serum, phagocytosis being active, the bacteria were brought into contact with substances within the leucocytes, which do not reach them in the normal leucocyte serum mixture, unless the leucocytes are disintegrated. Wallgren's (1899) observation that, in streptococcal peritonitis in the dog, there is a period of multiplication of the streptococci followed by extensive disintegration of leucocytes, and ensuing diminution of streptococci before the last ultimately get the upper hand, would fall in line with those observations. Weil (1913) got bactericidal effect with fresh serum (mouse) plus immune serum, plus healthy leucocytes; but neither with fresh nor immune serum separately, nor with leucocytes alone, nor in presence of immune and fresh serum, if the leucocytes had been frozen. He was working with the hypervirulent Aronson strain of streptococci. Bogendörfer (1921) points out that streptococci from erysipelas are less sensitive to the bactericidal action of leucocytic suspensions (empyema leucocytes) than the pneumococcus. Gay and Clark (1926) confirm Simon's result that washed leucocytes are superior to whole aleuronate exudate in bactericidal effect on streptococci, and they also show that acid extracts of the leucocytes after neutralization are more bactericidal than the leucocytes. Again, as in Simon's work, these extracts, although active against avirulent strains, are much less so against virulent ones. The acid bactericidal leucocytic extracts are heat-resistant up to 73° C.

In the last five years the part which the reticulo-endothelial system and the mobile clasmatocytes may play in streptococcal immunity has been investigated. Bass (1925) who was struck by the inability of large numbers of actively phagocytic leucocytes to control a streptococcal infection in a normal animal, and by the fact that streptococci injected to the pleura of a normal animal invaded the tissues widely before there was time for a leucocytic exudate to accumulate, whereas in an immune animal they did not, concludes that some other cell must be responsible for arresting their spread, and this he supposes to be the histiocyte. For the polymorphonuclear leucocyte he reserves the function of killing off the streptococci in foci from which the spread has been arrested by the fixed tissue cells, &c. The most extensive work on this subject is that of Gay and his collaborators already referred to. They studied the streptococcal infections of the pleura of rabbits previously treated with various sterile injections (starch, aleuronate, gum, broth, &c.) with a view to producing cellular exudate. They point out that an animal with an exudate in which clasmatocytes predominate is better protected than one with a predominantly polymorphonuclear exudate, and that a much larger percentage of the former cells are phagocytic ; further, that when an animal is surmounting an infection the exudate remains largely one of clasmatocytes, whereas when the animal is succumbing, it is the polymorphonuclears which accumulate. They conclude that the clasmatocyte is the important cell in immunity to streptococcus. They were unable, however, to show any considerable bactericidal power in clasmatocyte exudates *in vitro*, nor could they convey any passive protection by transferring exudate rich in clasmatocytes to a second animal. Further, the extension of their work indicates that the histological changes in the pleura which depended on the time which had elapsed between the injection of sterile aleuronate, &c., and introduction of the streptococci, was the controlling factor in these experiments. It appears to the writer that it would be a more reasonable explanation of these results to suppose that the clasmatocyte was the cell which disposed of streptococci when the body was less seriously threatened by them, either on account of relative insusceptibility to their toxic action, or on account of their isolation by a sufficient barrier of granulation tissue, whereas the polymorphonuclear leucocyte was called out to deal, often unsuccessfully, with a dangerous invasion.

Another aspect of antistreptococcal immunity which has received special attention in the last few years, is that of antitoxin formation. The inability to obtain toxic filtrates from streptococci in the early work, and then later the demonstration by Besredka (1901) Tchitchikine (1906), McLeod (1912), Braun (1912), and others that no antibody was formed to streptococcal hæmolysin, which is apparently a general toxin (McLeod and McNee, 1913), discouraged observations on this subject. The recent demonstration by the Dicks (1924[1]) of a streptococcal toxin of a different type capable of neutralization by antiserum has reawakened interest in

this subject. Thus, Birkhaug (1926) controls his treatment of erysipelas with antistreptococcal serum by determining whether the patient is still sensitive to a dose of erysipelas streptococcus skin toxin after the serum has been given. Andrewes (1925–6) brings forward some evidence to indicate that different streptococci produce different skin toxins. It is an open question whether such sera will eventually solve the therapy of advanced streptococcal lesions ; their failure in the suppurative complications of scarlet fever makes it doubtful.

All the evidence available goes to show that the property of protecting from streptococcal invasion which may be exhibited by the sera of immunized animals depends on the influence of the serum in rendering virulent streptococci suitable for phagocytosis by leucocytes and other cells. Whether this property is called immune opsonin with Wright, bacteriotropin with Neufeld and Rimpau (1905), anti-aggressin with Weil (1906), or antileukocidin with Nakayama (1920), it seems altogether probable that the process is one of neutralization of a toxin of the aggressin type. The failure of antisera to protect seriously, except when given prophylactically, may indicate that this substance cannot neutralize the streptococcal product *in vivo*, but prepares the cells so as to make them insusceptible. However that may be, the relationship of this type of streptococcal product or toxin to the thermostable skin toxin is one of the most interesting outstanding problems of streptococcal immunity.

Treatment.

Antistreptococcal Serum.

It is not intended to discuss here in detail the results obtained recently in the treatment of scarlet fever with antitoxic sera, as that will be considered below, nor, since it has already been considered, the theory of the action of antistreptococcal serum. Only the results which have been obtained in animal experiments and in the treatment of disease, and the methods of immunization which have given the best sera will be considered. This subject was reviewed in considerable detail by the writer in 1914, and the observations available up to then may be briefly summarized as follows : The early work of Marmorek (1895), Aronson (1902), and Besredka (1904), in which mice and rabbits were treated with the sera of horses immunized with hypervirulent passage strains, showed that prophylactic administration protected from many m.l.d., and that up to six hours after injection serum administration in large amount might cure an infection with 10 m.l.d. A few cures after the administration of larger amounts and at longer intervals from the time of infection are also recorded.

Antistreptococcal serum therapy was launched with a good deal of enthusiasm on account of these results : but the rapid accumulation of papers in which improved methods for producing antistreptococcal sera were described and defects of the older sera were pointed out, is evidence that the clinical results were not convincing (Moser, 1902 ; Menzer, 1902 ;

Tavel, 1903; Meyer and Ruppel, 1907; Marzer, 1910; Weil, 1913; Heidmann, 1912; Simon, 1907; Zangemeister, 1906, 1908). The line of criticism adopted by the last named is that Aronson's serum, while undoubtedly efficacious in protecting mice from various streptococci, is not necessarily equally effective in the human body. Aronson (1916), in reply, described protection of monkeys by antistreptococcal sera from a variety of animals, and he objected to Zangemeister's observations that prophylactic administration of serum to cases of infected uterine cancer before operation failed to protect from post-operative streptococcal infection on the ground that the action of serum depends on the co-operation of the leucocytes, which is lacking in cancerous subjects with deranged leucocytic mechanism. The derangement of the leucocytic system in this sense in cancer is, however, an assumption, whereas considerable intoxication of the leucocyte-producing tissues in severe streptococcal infections is altogether probable. The most obvious weakness in all this work is that in using highly susceptible animals, and strains of extreme virulence, the actual numbers of streptococci against which protection is exhibited is extremely small. It is not improbable that the m.l.d. for mice of some of these hypervirulent strains is at most 2 or 3 cocci, and that 10 m.l.d. of such strains, even if they multiply without any let or hindrance, will not have increased to a total of one million after 3 or 4 hours in the animal body. But when intervention with serum is attempted in severe streptococcal infections in man, the streptococci present in the tissues will already have developed marked virulence, i.e. they will resemble passage rather than stock strains, and they will be present in enormous numbers.

As a matter of fact when the protective powers of antistreptococcal sera have been tested on less susceptible animals, for which the m.l.d. of streptococci is numerically much larger, the results have been decidedly meagre. Thus, Weaver and Tunnicliff (1911), using the Aronson, the Pasteur Institute and Parke Davis & Co's antistreptococcal sera and working with guinea-pigs, record that protection is only obtained with difficulty, and that, when the serum is given a few hours after a small injection of culture. Brocq-Rousseu, Forgeot and Urbain (1925) could not do more than protect 9 out of 10 guinea-pigs by the prophylactic administration of 2 c.cm. of their serum if a subsequent injection of 2 to 5 c.cm. of culture was made, i.e. 1 to $2\frac{1}{2}$ m.l.d. (for mice, 0·0001 c.cm. of Aronson's strain represents 10,000 m.l.d.). Even in the rabbit when a 0·5 c.cm. dose of a passage strain, i.e. about 200 m.l.d. was given subcutaneously, and then treatment with large doses of either Parke Davis & Co., Burroughs Wellcome & Co. or Meyer and Ruppel's (Höchst Farbwerken) sera was commenced four hours later, no indication whatsoever of protection was observed, either in the sense of preservation of life, or of its prolongation beyond the period to which it might be prolonged by similar administration of normal serum. The serum of a horse immunized with the streptococcus used for infection gave no better results (McLeod, 1914).

The parts played by disappointing clinical results and by damaging experimental evidence respectively in producing the general disillusionment with regard to the value of antistreptococcal sera which existed at the opening of the 1914–18 War, are not easily determined, but it undoubtedly existed.

There is not, so far as I am aware, any published account of a clinical investigation of the value of antistreptococcal serum similar in its thoroughness to that by which the value of antidiphtheritic serum was established by Fibiger at the Blegsdam Hospital in Copenhagen, and it does not seem to be useful to review the numerous publications in which favourable or unfavourable opinions are expressed on insufficient evidence. Novak (1926) canvassed the opinion of 25 of the leading surgeons, obstetricians and gynæcologists in America, and got 16 unfavourable opinions, and 8 rather guarded statements that it might be useful as a prophylactic or when dealing with a strain to which it was entirely appropriate.

As regards methods of obtaining potent antisera, the emphasis in the early work was put especially on the development of high virulence in the strains used, and on the importance of introducing living or very slightly altered cocci. Schenck (1914) and Weil (1913) stress the importance of the introduction of live cocci, and Weaver and Tunnicliff (1908) point out the superiority of cocci killed by suspension in 25 per cent. galactose to those killed by heat for producing immunity. Neufeld (1903) is one of the few who have claimed immunizing qualities in heat-killed streptococcal suspensions. Recently Yoshioka (1922, 1923) in an extensive investigation of the conditions under which antistreptococcal and antipneumococcal sera are best prepared in the rabbit, emphasizes afresh the importance of high virulence in the strain used, and maintains that this is more important than whether the streptococcus is alive or dead. He got more potent sera by injection of heat-killed and highly virulent strains than with avirulent or slightly virulent live strains. Using these heat-killed highly virulent strains, and using small and very frequently repeated doses, he claims to have got more potent sera than any prepared previously. Part of this work has been followed up, confirmed and extended by Killian (1924), who, using mice, found short heating of the coccal suspension at about 100° C. gave the best results. Whether by modifying methods of inoculation and by increasing, along lines suggested by the work of the Dick's, the antitoxic properties of sera it may be possible to obtain in the future results superior to those of the past remains to be decided ; but the experimental work of the past may be summed up in the statement that at most it indicates a possible usefulness in antistreptococcal serum when it is administered as a prophylactic.

Vaccine Therapy.

In view of the fact that most of the work on vaccine therapy has been carried out with heat-killed vaccines administered subcutaneously,

and that suspensions of streptococci killed by heat have proved ineffective in producing immunity in the hands of competent observers (Lingelsheim, 1891), whereas others, Killian (1925) find subcutaneous injections valueless for producing immunity, it would not seem likely from *a priori* considerations that vaccine therapy would be successful. The reports of its value are conflicting, thus Richey (1919), working with human volunteers, found that he did not succeed by giving streptococcal vaccine in protecting them from tonsillitis following inoculation of streptococcal cultures on the fauces. Schorer (1907) observed that streptococcal vaccine administered in erysipelas invariably caused a rise in opsonic titre, but he could not definitely assign a prognostic significance to such rises, and he was unable to conclude very definitely in favour of vaccine therapy in this condition. Mironesco (1925), although he thought that the course of erysipelas was favourably influenced by vaccine therapy, noted that it did not prevent relapses. Goresco and Papesco (1925) who describe good results with Cantacuzène's polyvalent antistreptococcal serum in the same condition, discovered no value in stock streptococcal vaccine, either in accelerating cure or in preventing relapses. Ross and Johnson (1909), however, formed a general impression that vaccine has a marked value in erysipelas. In regard to scarlet fever, Weaver and Tunnicliff (1908), found no obvious advantage from heat-killed vaccine in preventing streptococcal complications, and Weaver and Boughton (1908), working with galactose-killed vaccine, failed to convince themselves of its value in erysipelas, or in preventing the onset of complications in scarlet fever. They did conclude, however, that where scarlatina was followed by rather chronic suppurative complications, such vaccines helped to clear them up rapidly. In connection with puerperal fever, distinctly good results are quoted by Western (1912) : of 28 cases with septicæmia treated by vaccine only, 15 died, whereas of 16 untreated cases, 14 died. The series is of course rather small to permit of very firm conclusions. Biermer (1925) reviews the literature dealing with prophylactic immunization in the puerperium, i.e. the combined use of serum and vaccine, and showed that the results are conflicting. He himself got no evidence at all favouring such procedure. Wolfsohn (1912), reviewed his results in the vaccine therapy of gonococcal, tubercular, staphylococcal and streptococcal lesions. Only chronic streptococcal lesions were considered. He concluded that the results of vaccine therapy were least convincing in the streptococcal lesions. There were only 20 per cent. of cases in which he could describe improvement or cure subsequent to treatment.

Many other papers on this subject might be quoted, but it seems obvious that in the more acute streptococcal manifestations at all events there is no convincing body of evidence in favour of vaccine therapy.

Role of Hæmolytic Streptococci in Human Disease.

By T. J. Mackie and D. G. S. McLachlan; with a Section by G. H. Percival.

Introduction.

The hæmolytic streptococci are responsible for inflammatory and suppurative conditions which vary greatly in their anatomical situation, severity and effects. Such lesions range from a trivial localized inflammation of transitory duration to one of great intensity, attended by marked suppuration and destruction of tissue, spreading rapidly in the tissue spaces and lymphatic channels, and followed by septicæmic or pyæmic infection of the blood. These organisms, in contrast with the staphylococci, show a remarkable capacity for spreading in the tissues, as illustrated in erysipelas, cellulitis, &c. In fact, a highly virulent strain may display its invasiveness by producing a fulminant septicæmia without any pronounced antecedent lesion at the site of initial infection. The severity of streptococcal infections depends not only on the invasiveness of the microbic strain, but also on the local and general resistance of the individual host. Compared with streptococci of the '*viridans*' type, hæmolytic streptococci are more rapidly invasive and produce lesions in which there is more pronounced suppuration and destruction of tissue. Thus, endocarditis due to these organisms is typically ulcerative and destructive as compared with the subacute form due to *S. viridans*. The toxicity of strains also varies, and, moreover, individuals show striking differences in their susceptibility to streptococcal toxins as apart from resistance to the actual organisms. This may, of course, be due to an acquired immunity resulting from previous infections or sub-infections. Thus, as is now generally believed, the scarlatinal syndrome (the rash and other general effects of scarlatina) is a manifestation of individual susceptibility to a diffusible toxin produced by many strains of hæmolytic streptococci.

The type of lesion with which these organisms are associated depends, therefore, on the avenue of infection, the tissue attacked, the virulence and toxigenic properties of the invading strain, and the degree of local and general resistance of the individual host. The diversity of pathogenic effects characteristic of this group of bacteria depends on such variable factors. It is of course possible that ' types ' of hæmolytic streptococci are highly specialized in their invasive and toxigenic properties, and that such conditions as erysipelas, scarlatina, &c., have almost a specific ætiology.

Hæmolytic streptococci may occur naturally as commensals (e.g. in the throat), and endogenous infection by such organisms may result under certain conditions. This is particularly true for secondary streptococcal infections. On the other hand, exogenous infection, associated sometimes with epidemic spread, is a conspicuous feature of certain diseases due to the hæmolytic streptococci.

Infection of the Skin.

Erysipelas.

Though an erysipelatous condition may on rare occasions be due to other organisms, true erysipelas is caused, in the great majority of cases, by hæmolytic streptococci, and illustrates the great tendency of these organisms to produce a spreading infection. Their multiplication in the lymphatic spaces of the cutis is attended by inflammatory œdema of the skin and adjoining tissues with infiltration of the spreading margin by mononuclear cells rather than by polymorphonuclear leucocytes, as is usual in other acute streptococcal lesions (MacCallum, 1920). Polymorphonuclear leucocytes are found in greater numbers, however, in the more advanced lesions and occasionally the accumulation of pus cells may cause the formation of cutaneous abscesses. As shown by Fehleisen (1883), the streptococci are most numerous at the periphery of the lesion, and can be readily isolated by excising portions of skin at the spreading edge. Birkhaug (1925[2]) has utilized, however, a simpler method of obtaining the organisms, viz. by injecting a small quantity of sterile saline, e.g. 0·5 c.cm. into the skin at the edge of the lesion and aspirating fluid from the resulting wheal.

By the injection of pure cultures, Fehleisen was able to reproduce the disease in man and animals, and for a time '*S. erysipelatis*' was regarded as a distinct species differentiated from *S. pyogenes* by certain cultural characteristics and by a supposed specific pathogenicity. Later investigators, however, showed that there was no essential difference between these organisms, and that streptococci from a variety of sources might produce erysipelas in animals provided the virulence of the strains employed was sufficiently exalted by passage. The same strain of streptococcus might thus at different times produce suppuration, erysipelas or septicæmia, variations in the virulence of the organism and in the susceptibility of the animal modifying the nature of the pathological condition produced. These experimental findings are also in agreement with clinical experience. Though erysipelas does not as a rule complicate other streptococcal conditions, it may occasionally develop, e.g. during scarlet fever (Appiani, 1908), after operations on the nose and infected nasal sinuses (Holmes, 1907; Parry, 1911), and after angina (de la Chapelle, 1908). In this connection it is of interest to record the experiment of Dick and Dick (1927) who produced a simple inflammation of the throat in 3 out of 5 volunteers by swabbing their tonsils with cultures of hæmolytic streptococci isolated from cases of erysipelas. During the War, erysipelas was not infrequently a complication of infected wounds (Douglas, Fleming and Colebrook, 1920).

The clinical observation that erysipelas passes as such from case to case has led to the suggestion that erysipelas strains represent streptococci with a fixed grade of virulence, but it is to be noted that these organisms can act differently under different conditions, as is shown by the intimate ætiological relationship which undoubtedly exists between erysipelas and

puerperal fever (see Ker, 1920). The further clinical observations that erysipelas, especially of the face and scalp, may be associated with small cutaneous abscesses, while the severe forms may lead to streptococcal infection of other organs indicate that there is no essential difference in the pathogenic powers of the streptococci causing erysipelas and those associated with other pyogenic processes.

In erysipelas, infection is believed to take place through a breach of the integument, though in the so-called idiopathic form there is no observable external abrasion. Transmission by fomites and by the hands of attendants was common in surgical work before the introduction of antiseptic methods. Facial erysipelas, the commonest variety, generally begins near the muco-cutaneous junction of the nostrils and many cases present a history of preceding coryza (Erdman, 1913). Holmes (1907) in an interesting discussion on the source of infection in erysipelas, emphasizes the frequency of facial erysipelas starting in the neighbourhood of the nose ; he regards the nasal passages and sinuses as sources of the infecting agent. Contagiousness is slight according to Erdman (1913), who could trace only 3 cases of family infection in a series of 800 patients. On the other hand, some observers have reported several instances of contact infection from cases of erysipelas and consider complete isolation necessary (Redlich and Krasso, 1926).

The literature dealing with erysipelas has been reviewed by de Marbaix (1892), and Gay and Rhodes (1922).

In recent years the question has arisen whether the hæmolytic streptococci from erysipelas constitute a distinct serological type. The observations of Tunnicliff (1920[2]) and of Eagles (1924, 1926) would indicate that this is the case. According to Birkhaug (1925[2]), 91·2 per cent. of the erysipelas strains which he studied could be distinguished by agglutination tests from strains obtained from scarlatina, puerperal fever, tonsillitis and other sources. Stevens and Dochez (1926[1 & 2]) concluded that erysipelas strains possess a characteristic group agglutinogen, which is not, however, strictly specific for strains from that condition.

The results of the above workers would suggest that hæmolytic streptococci from erysipelas constitute a distinct serological group, but it is evident that there is no considerable fraction in the antigenic structure of erysipelas streptococci by which they can be distinguished from other hæmolytic streptococci, and it cannot be claimed that erysipelas streptococci form a serological group analogous to, say Type I pneumococcus. Other investigators have been unable to classify hæmolytic streptococci from different conditions in serological groups corresponding to the source of the strains (vide p. 93), and it seems improbable that erysipelas strains are exceptional in this respect.

Further evidence regarding the unity of erysipelas streptococci has been adduced by Schwartzmann (1927), who found that 16 out of 21 erysipelas strains were lysed by a streptococcus bacteriophage, while 64 strains from other sources were unaffected.

Hæmolytic streptococci from erysipelas have been found to possess toxigenic properties analogous to those of the scarlatina strains, and the effect of intracutaneous injection in man with filtrates of fluid cultures has been studied by Birkhaug (1926[1]), Eagles (1926), Okell and Parish (1928) and others. Birkhaug in particular has utilized a skin reaction similar to the Dick test for determining the susceptibility of individuals. He found that 27 per cent. of adults and 21 per cent. of school children reacted positively, and concluded that the toxin differs specifically from that produced by scarlatina strains, since the reaction was neutralized by convalescent and immune erysipelas antisera, but not by scarlatinal antitoxin. Parallel tests with filtrates from scarlatina and erysipelas strains showed that a greater number of individuals reacted with the scarlatina toxin. Okell and Parish (1928), however, were unable to confirm the dissimilarity of the toxins of erysipelas and scarlatina streptococci, and, indeed, could find no specific difference among toxins produced by hæmolytic streptococci of diverse origin.

Erysipelas is usually self-limiting and some form of increased resistance must occur when recovery ensues, but clinical experience has indicated that no lasting immunity results from an attack, and recurrence is observed in about 10 per cent. of cases (Gay and Rhodes, 1922). Observations on the experimental disease in rabbits, and experimental inoculation of human beings have thrown some light on the nature of immunity to erysipelas. Thus, Fehleisen (1883) succeeded in producing erysipelas in 6 out of 7 patients suffering from malignant disease by injection of pure cultures of '*S. erysipelatis*,' but reinoculation of 2 of these 6 patients, 3 to 4 weeks later with a culture of the same organism was ineffective. Fehleisen interpreted these observations as indicating transient immunity, and later observers have confirmed the fact that some degree of immunity follows infection. Meierowitsch (1887) found that rabbits which recovered from experimental erysipelas showed increased resistance to reinfection of the skin. Cobbett and Melsome (1896) observed that an absolute but short-lived local immunity was conferred upon the area of skin originally affected, and that recovery from erysipelas was also associated with general cutaneous immunity which was inconstant in degree.

The question of local immunity of the skin as contrasted with general immunity has been investigated by Gay and Rhodes (1922), who found that recovery of rabbits from erysipelas was associated with the development of general cutaneous insusceptibility for a period of at least three months. Such rabbits, however, could still be infected by intravenous injection of the streptococcus. On the other hand, intravenous immunization protected better against subsequent intravenous than against intradermal infection. Amoss and Bliss (1927) have shown that after the healing of experimental erysipelas in rabbits the area of skin involved shows greatly increased resistance to reinfection, which extends in the direction of the efferent lymphatics. Other areas only become insusceptible when a humoral immunity develops.

Rivers and Tillett (1925) and Birkhaug (1926[1]) have reported that local passive immunity can be conferred on an area of the rabbit's skin by intradermal injection of antistreptococcus and antitoxic sera respectively. A certain degree of non-specific cutaneous immunity, however, follows intradermal injection of broth or normal rabbit serum (Rivers and Tillett, 1925) or inflammation of the skin caused by the application of oil of mustard (Cobbett and Melsome, 1898). Parish and Okell (1928), moreover, have been unable to find any evidence that antistreptococcal or antitoxic sera exert a specific inhibitory action on the development of skin reactions in rabbits following intradermal injection of hæmolytic streptococci. Experimental evidence on the value of immune sera in preventing the development of erysipelas-like lesions in rabbits is therefore inconclusive.

Similarly, treatment of persons suffering from erysipelas by specific antitoxic or antibacterial sera has given conflicting results, a beneficial effect being reported by Birkhaug (1926[3]) and others, while McCann (1928) has been unable to confirm this (see also Francis, 1928).

The observations of Birkhaug (1926[1 & 3], 1927) and Singer and Kaplan (1926) would suggest that immunity to erysipelas is antitoxic in nature, since patients in the early stages of the disease give a positive reaction on intradermal inoculation of filtrates of erysipelas strains, while they react negatively at a later stage. McCann (1928), however, could find no relationship between the skin reaction and the stage of the disease.

While there is, therefore, a considerable amount of evidence in favour of the development of antitoxic substances in the serum of persons convalescent from erysipelas, the validity of the intradermal reaction as an index of immunity in erysipelas cannot yet be regarded as proved, and the importance of a general antitoxic immunity in relation to the resistance of the skin has still to be determined.

Impetigo and other Streptococcal Infections of the Skin.

By G. H. Percival.

Within recent years many investigations have been carried out in an endeavour to establish the ætiological significance of streptococci in various skin diseases. The results are still somewhat contradictory, and a satisfactory solution for most of the problems involved has still to be found. There are several sources of error which must be taken into consideration when an attempt is made to evaluate the importance of a specific organism in the causation of a superficial skin lesion. The skin surface is frequently contaminated with micro-organisms and is constantly open to further contamination, so that any organism cultivated from it may have existed there merely as a commensal. In a lesion which has become secondarily infected, the contaminating species may completely outgrow the primary organism, so that the presence of the latter may be overlooked.

The ordinary cultural methods are unsatisfactory for the isolation of a streptococcus from the skin, on account of the ease with which this

organism is overgrown by others, especially staphylococci. Two selective methods are available which to some extent overcome this difficulty. Haxthausen's method (1927) consists in employing an ordinary bouillon medium containing 1 : 100,000 to 1 : 500,000 crystal violet, the latter substance inhibiting the growth of staphylococci. By inoculation of material on solid media containing crystal violet some indication may be given as to the degree of infection present, according to the number of colonies obtained.

In Sabouraud's method (1892, 1900), the serous exudate or an emulsion of scales from the lesion is cultured in ascitic serum or bouillon in a capillary pipette. This arrangement allows of aerobic conditions in the wide part of the pipette, and semi-anaerobic conditions in the capillary stem—the latter environment being favourable to streptococcal growth whilst unfavourable to the multiplication of staphylococci. At the end of eight hours the fluid in the stem of the pipette is examined and subcultured. In this way it is possible to obtain pure cultures of streptococci from mixed infections.

Occurrence of streptococci in normal skin.

Frederic (1901) who used Sabouraud's technique, obtained cultures of streptococci from the skin of 7·5 per cent. of normal individuals. Flehme (1920) records positive cultures from the skin of 15 per cent. of healthy persons. Haxthausen's figures (1927) are 7·5 per cent. positive in normal persons, and 15 per cent. in cases suffering from various non-impetiginous dermatoses ; in both classes the primary cultures on solid crystal violet media showed very scanty colonies.

Impetigo contagiosa and ecthyma.

Impetigo was first described as a clinical entity by Tilbury Fox in 1864. Crocker (1881) was the first to suggest its micrococcal origin and to discover the presence of chained organisms in the fluid obtained from unruptured bullæ. Bockhart in 1887 concluded, however, that impetigo contagiosa was due to a superficial staphylococcal infection. In 1893, Leroux cultured several varieties of streptococci and staphylococci from the unbroken bullæ in 70 out of 120 children suffering from impetigo contagiosa. He reproduced the disease experimentally by inoculating the isolated organisms and obtained only streptococci on reculturing material from the experimental lesions. Balzer and Griffon (1897) confirmed the work of Leroux, and obtained streptococci in pure culture from the bullæ in 31 cases of impetigo contagiosa and in 14 cases of ecthyma.

In 1900, Sabouraud divided impetigo into two distinct groups, that of Bockhart, which was really a folliculitis due to *Staphylococcus aureus*, and that of Tilbury Fox due to a streptococcus. Dubreuilh and Brandeis (1910), in repeating Sabouraud's work, examined a series of cases of impetigo contagiosa, in which they noted the presence of both streptococci

and staphylococci, using Sabouraud's method. Although the streptococci predominated, they remark that the almost constant occurrence of secondary infections renders the conclusions which can be drawn from cultures very uncertain. Since then, however, the view that impetigo contagiosa is due to a streptococcal infection has been generally accepted, and recent work involving the employment of more selective cultural methods has verified this opinion (Farley and Knowles, 1921; Haxthausen, 1927; Sabouraud, 1928; and others). The disease is apparently not due to a single specific type though hæmolytic streptococci are the most frequent varieties present. Balzer and Griffon isolated *S. pyogenes* (vide *supra*); Flehme found *S. longus* in all his cases; and Gilchrist (1900) identified *S. pyogenes* in 3 cases. In 24 cases examined, Farley and Knowles (1921), using Holman's classification, found 10 to be due to *S. pyogenes*, 11 to *S. anginosus*, 2 to *S. subacidus* and 1 to *S. fæcalis*. They also state that *S. fæcalis* was a frequent infecting agent in impetigo contagiosa during the War. They concluded that with proper precautions and under proper conditions streptococci may be obtained from nearly all cases of impetigo contagiosa. Smith and Burky (1924) isolated hæmolytic streptococci from 6 out of 9 cases of impetigo contagiosa and identified *S. pyogenes* in 4 cases, and *S. infrequens* (Holman, 1916) in the remainder.

Virulence of organism. Balzer and Griffon (1897) found the streptococci obtained by them from cases of impetigo contagiosa to be pathogenic to animals, causing erysipelas, abscess formation, and fatal septicæmia. Smith and Burky (1924) state that the organisms isolated by them were of low virulence, but that by passage through mice the virulence was increased twenty times. Sabouraud (1928) mentions that streptococci isolated from cases of impetigo contagiosa cause a fatal septicæmia when injected intravenously in rabbits.

From the foregoing it must be concluded that there is no specific streptococcus of impetigo, and this fact has prompted the suggestion (Smith and Burky) that the streptococci are merely secondary invaders. There is, however, no positive evidence of the presence of any other specific factor, while inoculation experiments with the isolated streptococci have many times given rise to typical impetigo lesions. In all probability a suitable 'soil' renders infection more liable to occur, and is specially important in the transformation of the ordinary impetigo lesions into those of ecthyma. Frequently recurring attacks of impetigo contagiosa in the same patient suggest that no immunity to the disease is acquired (see Smith and Burky, 1924). Relapses in individual cases are probably due to the persistence of streptococci in chronic perionychia, in minute labial or nasal fissures, in slight retro-auricular lesions, or in the scalp.

The superficial nature of the lesions and the ease with which they can be cured argue in favour of an infection with an organism of low virulence, and this has been borne out by some of the results of animal inoculation

(vide *supra*). The rarity of erysipelas or cellulitis as a complication of impetigo contagiosa also suggests that the streptococcus responsible for the latter disease is not of a virulent nature.

Pemphigus neonatorum.

Knowles and Munson (1923) examined six cases of bulbous impetigo in infants, and obtained pure cultures of *Staphylococcus aureus*. McCandlish (1925) made cultures from a considerable number of cases in a series of 224 infants suffering from this type of impetigo and states ' that for the most part the *Staphylococcus pyogenes aureus* was found '. Cole and Ruh (1914) and Falls (1917) found staphylococci in pure culture in 9 and 54 cases respectively. More recently Haxthausen (1927), using Sabouraud's technique, cultured streptococci from the lesions, but when cultures were made directly on to solid media containing crystal violet, the growth of streptococci was extremely scanty.

Contrary to the majority of workers, Sabouraud (1928) holds that pemphigus neonatorum and impetigo contagiosa are one and the same disease ætiologically, as he has succeeded in isolating streptococci from cases of pemphigus neonatorum. Since the disease commences almost invariably on the buttocks he suggests that the infection is derived from the bowel contents. The skin reaction in pemphigus neonatorum consists essentially of a serous exudation which results in the formation of a bulla. This type of reaction is characteristic of acute superficial streptococcal infections of the skin, and is in marked contrast to the usual reaction to a staphylococcal infection, which is a localized cellular infiltration.

The intertrigos.

The work of the French dermatologists, and especially that of Sabouraud, has shown that streptococci are frequently responsible for various types of intertrigo. He remarks (1923) that a streptococcal lesion commences by the separation of a superficial epidermal layer, extends without infecting the follicles, and has a predilection for the folds. Haxthausen has recently corroborated this work. Sabouraud considers the following lesions are frequently due to this type of infection : retro-auricular intertrigo, often associated with ' dartres volantes ' on the face, and frequently referred to as a manifestation of ' seborrhœic dermatitis ' : periaural dermatitis, associated with fissures at the corners of the lips (perlèche) and fissures and dermatitis of the nasal folds. Intertrigo of the ano-genital region is stated to be of streptococcal origin by Sabouraud, a view which is also held by Murray and Chipman. Murray (1918) considers that the infection may be primary, or secondary and aggravating, and if the latter the primary cause may have passed away. He found streptococci in 94 per cent. of cases (1916), and concludes that the beneficial results and cures obtained by vaccine treatment justify the belief in the infective ætiology of pruritus of the anus and genitals. Chipman (1916) found *S. fæcalis* alone or associated with *B. coli* in 60 per cent. of cases of intertrigo of the anal fold.

Seborrhœa corporis (*eczematide, Darier ; parakeratosis psoriasiformis, Brocq*).

This condition was first recognized by Unna as being definitely associated with ' eczema seborrhœica ' of the scalp. The histological picture is that of eczematization. Sabouraud (1928) has isolated streptococci from identical lesions, and has distinguished these organisms in sections. Clinically he traces all stages of the condition from an ordinary impetigo to a typical patch of seborrhœa corporis. Milian (1928), quoted by Sabouraud (1928), has reported a case of seborrhœic dermatitis of the scalp associated with dry scaly lesions on the body, from numerous areas of which he was able to isolate streptococci. Haxthausen found a heavy infection of streptococci associated with some of the cases of seborrhœa corporis which he examined. These writers conclude that a proportion of cases are streptococcal, while others are staphylococcal.

A point of practical interest is that the pathological process initiated as a result of these external infections consists of an eczema reaction, so that local organismal infection must be recognized as a possible eczematogenic factor (Sabouraud, 1928 ; Civatte, 1924). Although the characteristics of the streptococci isolated from these lesions have not yet been investigated, it is very probable, from comparison with the hæmolytic streptococci isolated from impetigo, that the organisms in question are capable of elaborating a diffusible toxin ; if this is the case, the eczema reaction which is provoked may be due to the direct action of such toxin. This supposition would serve to explain the eczematous process, and would be in line with the modern conception of eczema, which postulates a reaction between an external chemical substance and sensitized epidermal cells. It is always open to question, however, whether such cases are primarily non-infective eczemas which have become impetiginized secondarily, or whether the organism isolated has produced the eczematization.

The difference between impetigo contagiosa, which usually runs a rapid course, and the persistently chronic character of some streptococcal infections may be due to variations in the type or virulence of the infecting organisms—points on which there appears to have been little or no work done—or to variations in the host and the reactive powers of the tissues. Alterations in the skin secretion by facilitating the growth of the organisms may be responsible for the persistence of the infection. Usher (1928) has demonstrated this in connection with the sugar content of the sweat and the growth of staphylococci.

Paronychia.

Streptococci frequently cause an acute impetiginous lesion of the nail fold, either alone or in association with lesions elsewhere. The condition is probably responsible in many cases for the spread of the disease to other exposed parts as a result of auto-inoculation. Occasionally the local infection is chronic from the outset. Haden and Jordan (1925) and Sutton (1926) report such cases of paronychia associated with dental

infection, in which they have isolated streptococci from both sources. Morrow and Lee (1915) made cultures from 16 cases of chronic paronychia and isolated *Staphylococcus albus* in pure culture in all cases. They made primary inoculations on serum-agar, a technique which undoubtedly explains their inability to demonstrate the presence of streptococci.

G. H. P.

Infection of Wounds, Subcutaneous Tissue, Lymphatics; Bones, Joints; Septicæmia and Pyæmia.

Streptococcal infection of the subcutaneous and submucous connective tissues may be localized in character, or, as is frequently the case, may tend to spread through the tissue spaces and along the lymphatic vessels of the area. The severity of the infection bears no necessary relation to the nature of the wound which allows these organisms access to the tissue : thus, even a slight abrasion or a pin-prick may open the way to a serious infection. In pre-antiseptic surgery many of the septic complications of operations were undoubtedly caused by streptococci as judged by the nature of the inflammatory and suppurative processes described.

Infection of Wounds, Subcutaneous Tissue and Lymphatics.

Streptococci are not infrequently present on normal skin—Haxthausen (1927) isolated them in 7·5 per cent. of a series of cases—and thus have ready access to the tissues when there is a breach in continuity of the skin or even of the epidermis.

' Clean ', healing wounds that show no evidence of microbic infection may harbour micrococci and other bacteria (Cheyne, 1882 ; Dudgeon and Sargent, 1906), and the knowledge that micro-organisms might be present in healthy as well as suppurating wounds delayed recognition of their pathogenic action (see Cheyne, 1882).

During the War, conditions were particularly favourable for the infection of wounds. The skin and clothing of troops in the trenches were inevitably soiled with mud teeming with micro-organisms of intestinal origin, and the initial contamination of war wounds was, therefore, largely due to fæcal organisms (Fleming, 1915), the most important being *B. welchii*, *Enterococcus*, *S. fæcalis*, staphylococci and *B. coli*. Though often not initially present *S. pyogenes* was found in practically every septic wound and was responsible for many of the complications and sequelæ, e.g. erysipelas, persistent suppurating sinuses, &c. (Douglas, Fleming and Colebrook, 1920). The importance of streptococci, especially hæmolytic streptococci, in war wounds was recognized by Levaditi (1918[1]) who drew attention to their action in delaying healing. Bunce, Berlin and Lawrence (1919) found them in 24 per cent. of a series of 985 wounds, and regarded them as the chief cause of the failure of secondary suture.

The suggestion has been made that different races vary in their susceptibility to streptococcal infections. Thus, it is stated (Lingelsheim, 1928) that with equally frequent occurrence of streptococci on the skin, only 19 per cent. of wounds among Belgians were infected in comparison with 56 per cent. of wounds in British troops.

Circumscribed *abscesses* in the subcutaneous tissue are sometimes produced by streptococci though they are more commonly due to staphylococci ; in some cases the two organisms may be associated. Zuckermann (1887) collected the results of a number of different observers and reported that the staphylococcus was present in 71 per cent. of 495 abscesses, the streptococcus in 16 per cent., the two organisms together in 5·5 per cent., while other pyogenic organisms occurred less commonly.

The condition of *cellulitis* is generally due to hæmolytic streptococci which have gained access to and are spreading in the subcutaneous tissue or other connective tissue zones. Deep infected wounds where drainage is deficient are particularly liable to be followed by this condition. Cellulitis is most likely to develop in areas where there is much loose connective tissue traversed by lymphatic vessels draining an infected surface wound ; e.g. pelvic cellulitis may occur in association with puerperal infection of the uterus (vide p. 97) ; cellulitis of the axilla may follow on an infected wound of the hand. The loose connective tissue offers much better opportunities for the development of streptococci than the dense corium, and the free lymphatic circulation aids the rapid spread through the tissue spaces. The tendency of streptococci to produce this type of infection was noted by Ogston (1881, 1882) and Rosenbach (1884).

Acute inflammation of the peripheral lymphatic vessels (*lymphangitis*) manifests the spread of micro-organisms, generally hæmolytic streptococci, from a septic focus in the skin, subcutaneous or submucous tissues. The organisms multiply in the lymphatic vessels and coagulation of the lymph may occur. Wavy red lines in the skin indicate the course of the inflamed superficial lymph vessels, and abscess formation may occur in the path of these vessels or in the regional lymph glands. The formation of these abscesses may be due to associated staphylococci (Fischer and Levy, 1893). Lymphangitis, as noted by Verneuil and Clado (1889), presents certain analogies with erysipelas. In both, lymphatic vessels are affected ; in erysipelas the small lymphatic vessels in the corium are the seat of the inflammatory process, in lymphangitis the larger lymph vessels in the deeper parts of the skin and in the subcutaneous tissue are involved.

Acute *lymphadenitis* may be due to a variety of bacteria and varies in severity with the type and virulence of the infecting organisms. Infection of the lymphatic glands occurs by way of the afferent lymphatic vessels, which may show no evidence of inflammation. The primary focus may be in the skin, subcutaneous tissue, mucous membranes or other area. Lymphadenitis associated with hæmolytic streptococcus infections may be mild in character or may be very acute. Thus, in simple tonsillitis or

in scarlet fever the cervical lymph glands may be only slightly inflamed ; on the other hand, acute inflammation and abscess formation may occur from infection with hæmolytic streptococci in severe diphtheritic or scarlatinal inflammation of the throat.

Infection of Bones and Joints.

While acute suppurative periostitis and osteomyelitis are usually due to *Staphylococcus aureus*, cases are occasionally noted in which a hæmolytic streptococcus is the associated organism. Gonser (1902) collected from the literature 27 cases of streptococcal osteomyelitis and himself reported 32 cases of osteomyelitis, one of which was due to a streptococcus. Trendel (1904) recorded 88 cases, of which 77 were caused by *Staphylococcus aureus* and 5 by *S. pyogenes ;* in 5 of this series both organisms were present. Starr (1922) has also described a case of osteomyelitis due to a hæmolytic streptococcus. Pyæmia may result with metastatic abscesses in various organs, and suppurative arthritis may occur from extension of the process from the bone-marrow into a neighbouring joint. The atrium of infection is frequently obscure, and conditions of this nature are often referred to as ' cryptogenetic ' infections.

Acute suppurative *arthritis* is frequently part of a systemic infection and may occur in ulcerative endocarditis, erysipelas, puerperal fever, or may complicate the specific fevers, e.g. scarlatina. In other cases the source of infection is obscure. As noted by Harris (1925) streptococcal arthritis varies in severity more than staphylococcal infections. Scarlatinal arthritis is generally mild (Joe, 1924) ; sometimes it may be suppurative (Osler and Macrae, 1920). Harris investigated 51 cases of suppurative arthritis in children and demonstrated *Staphylococcus aureus* on 24 occasions ; hæmolytic streptococci were isolated from 15 cases, but non-hæmolytic types were not encountered. In 12 of these 15 cases infection had occurred by the blood-stream and in 8 there was an obvious primary focus of infection (e.g. otitis media, scarlatinal angina). Acute suppurative arthritis due to hæmolytic streptococci may result from spread of an infective process in an adjoining long bone (Starr, 1922).

The literature dealing with streptococcal infection of joints has recently been reviewed by Thomson (1929).

Septicæmia and Pyæmia.

Localized inflammatory lesions due to hæmolytic streptococci may at any time be complicated by the development of a general infection, the organisms gaining access to, and multiplying in, the blood-stream. Septicæmia is particularly liable to develop when extremely virulent streptococci are introduced into the tissues. This is well exemplified by the infection of trivial accidental wounds contracted by surgeons while operating on septic cases and by pathologists in the course of post-mortem examinations. In such cases there may be only an insignificant reaction at the point of entry of the organism and no lymphangitis or lymphadenitis (Kolle and Hetsch, 1922). A general blood infection may result from

various streptococcal conditions, e.g. cellulitis (Rosenbach, 1884) puerperal sepsis (vide p. 97), scarlatina (Thomson, 1924), erysipelas (Pfuhl, 1892).

As first described by Rosenbach (1884), streptococci may cause a pyæmic condition, the organisms giving rise to metastatic abscesses in various organs of the body. In the majority of Rosenbach's cases the development of secondary foci apparently followed septic embolism from infected operations wounds. According to Kolle and Hetsch (1922), a pyæmic condition may occur in 20 to 25 per cent. of cases of streptococcal septicæmia, secondary foci being particularly common in the lungs and joints. Pyæmia is, however, more frequently associated with staphylococcal infections.

A serological study of the hæmolytic streptococci occurring in suppurative processes has been made in recent years by various workers. Thus, Gordon (1921) reported that the majority of strains from pyogenic conditions (abscess, cellulitis, arthritis, osteomyelitis, meningitis, &c.) could be classified in one group on a basis of agglutinin-absorption tests, while Kinsella and Swift (1918) concluded from the results of complement-fixation experiments that hæmolytic streptococci form a homogeneous group. Douglas, Fleming and Colebrook (1920) found that 24 strains of *S. pyogenes* isolated from war wounds belonged to the same serological type as judged by agglutination and agglutinin-absorption tests. These observations have not been confirmed by later work, and it is doubtful whether strains derived from suppurative conditions are identical serologically. This question is discussed in the sections dealing with scarlatina, erysipelas and puerperal fever.

Infection of the Upper Respiratory Passages, Otitis, Pneumonia and Empyema.

Infection of the Upper Respiratory Passages.

Though hæmolytic streptococci are infrequent relatively to the non-hæmolytic types, in the mouth, throat and nose of healthy persons, they may nevertheless occur in these situations, especially in the pharynx. Pilot and Davis (1919) found these organisms in the throat secretion in 61 per cent. of persons examined ; in excised tonsils they were present (in large numbers) in 97 per cent. ; they were less frequently found in the throat after tonsillectomy. According to Pilot and Pearlman (1921), hæmolytic streptococci are exceedingly common in excised tonsils and adenoids. They regard these structures as foci in which such organisms flourish. Eves and Watson (1925) suggested that the crypts of the tonsils are their natural habitat. The writers noted the presence of hæmolytic streptococci in the throats of 20 of 55 apparently healthy persons, i.e. 36 per cent. According to Fox and Stone (1927) the occurrence of hæmolytic streptococci in the throat has a relationship to local pathological conditions (either as cause or effect), but they are sometimes unassociated with any evident lesion. It is apparent,

therefore, that hæmolytic streptococci occur as commensals in the throat, being more frequent and more numerous in association with a local pathological process.

Hæmolytic streptococci are often associated with the more severe forms of acute tonsillitis, pharyngitis and rhinitis. Local suppuration and abscess formation may result, e.g., tonsillar abscess, and from such initial lesions the infection may also spread in various directions, e.g., to the lymph glands of the neck, into the accessory nasal sinuses, and by the Eustachian tube to the middle ear (vide *infra*). The so-called Ludwig's angina is usually due to these organisms ; it is a cellulitis of the submaxillary region of the neck arising from a primary focus in the mouth or throat. The relationship of acute streptococcal infection of the throat to scarlatina is discussed later (p. 87). Reference is also made to the possibility of other forms of streptococcal infection being due to the transmission of streptococci from inflammatory conditions of the throat (pp. 82, 99). Blood infection may arise from a primary infection of the throat resulting in septicæmia or metastatic lesions in other parts of the body (vide *infra*). Hæmolytic streptococci are frequent secondary invaders of the throat, following in the wake of some other infection, e.g., diphtheria. It may be assumed that both endogenous and exogenous infection of the throat by these organisms occur ; a strain of low virulence may only invade the tissues as a result of previous weakening of the local or general resistance, while a highly virulent strain may pass from one person to another, reproducing in each successive host an active infection. Sequelæ resulting from streptococcal infection of the throat are erysipelas, otitis and mastoiditis, suppuration in the maxillary antrum, peritonitis, endocarditis, pneumonia and pyæmia (see de la Chapelle, 1908).

Many epidemics of ' sore throat ' have been recorded in the literature as traceable to milk (see Savage, 1912 ; Winslow, 1912 ; Capps and Miller, 1912 ; Davis and Rosenow, 1912). Hæmolytic streptococci have been isolated from such epidemic cases, and milk has been incriminated by the finding of the same organism in it, and the fact that the cows from which it was obtained were suffering at the time from a streptococcal mastitis. The angina in such cases has often assumed a severe form with intense inflammation, a grey fibrinous exudate on the mucosa, and cervical adenitis, and has in some cases been followed by pneumonia, peritonitis, metastatic lesions or septicæmia. Besides cases traceable to milk supplies, contact infection has also contributed to such outbreaks. The infection has not been invariably related to udder disease, and the question has arisen whether the contamination of milk might be due to carriers. On the other hand, the massive nature of the infection and its continuous character has undoubtedly indicated udder disease as the source of the contamination of the incriminated milk. Smith and Brown (1915) and Krumwiede and Valentine (1915) from their studies of these outbreaks concluded that the udder infection in question originates from human contact (e.g. from a person with a septic throat), and that the organism

invades the milk ducts and multiplies in the udder without necessarily causing a mastitis. According to Mathers (1916), the streptococci of bovine mastitis have no relationship to sore throat in the human subject.

Brown, Frost and Shaw (1926) have described a capsulated type of hæmolytic streptococcus found in persistent udder infection related to these outbreaks. They suggest that the organism is primarily of human origin, and that the udder is infected from a human source. A recent contribution to our knowledge of this subject bearing on the origin of milk-borne scarlatinal infection is discussed later (p. 90).

Streptococci are less common in suppuration of the accessory sinuses of the nose than pneumococci. Extension of infection may take place from these sinuses to the central nervous system. This has been specially studied by Turner and Reynolds (1926, 1927, 1928) in post-mortem specimens from 20 cases. The actual nature of the infection was not inquired into in all cases, but their series included streptococcal infections. They were able to show that the infection spread in most cases directly through the osseous wall of the air sinus (17 cases). In one instance, the route of extension was by the perineural lymph sheath of the olfactory nerve. In two cases the cavernous sinus became infected from the sphenoidal air sinus by way of the perforating veins.

Attention has been drawn more particularly in recent years to the possibility of virulent hæmolytic streptococci being introduced into operation wounds from the nose and throat of surgeons and those in attendance at operations (see Meleney and Stevens, 1926; Meleney, 1927). Puerperal infection may arise in an analogous way (vide p. 98). This possibility should be obviated by masking.

Otitis Media.

Otitis media usually occurs as a result of an ascending infection from the nasopharynx. Various pyogenic organisms may produce it, but a considerable proportion of cases are due to hæmolytic streptococci either alone or associated with other organisms. Wirth (1926) recorded the presence of these organisms in 53·8 per cent. of cases; it may be noted that in his series, *S. mucosus* was found in 23·8 per cent. and pneumococci in 7·1 per cent. '*S. viridans*' was infrequent (less than 1 per cent. of cases). According to Abrahams and Bonoff (1925), *S. mucosus* is more frequent in this condition than is generally believed and especially in middle-aged and elderly persons; they have drawn attention to the affinity of this organism for bone, the widespread destruction produced by it, and the resulting intracranial complications.

Hæmolytic streptococci are also found in the various complications of otitis, viz. mastoid empyema, sinus thrombosis, meningitis and intracranial abscess.

Pneumonia; Empyema.

Hæmolytic streptococci are rare in cases of acute lobar pneumonia. Of 529 cases recorded by Avery, Chickering, Cole and Dochez (1916),

S. pyogenes was associated with 13 only. In broncho-pneumonia, on the other hand, the frequency of hæmolytic streptococci is much greater. This refers both to primary broncho-pneumonic infection and also the broncho-pneumonia occurring as a complication or sequela of influenza, measles, diphtheria, whooping-cough, &c. In the latter group the primary virus predisposes to secondary invasion by various organisms including the hæmolytic streptococci. One of the earliest bacteriological surveys of broncho-pneumonia was that of Eyre (1909), who showed that streptococci were almost as frequent in primary and secondary broncho-pneumonia as the pneumococcus. The streptococci found were classified as '*S. longus*'. Thus, among 86 cases in which pure cultures were obtained, pneumococci were found in 33, streptococci in 32, *Staphylococcus aureus* in 10; of 78 cases with mixed infections, pneumococci were present in 46, streptococci in 33 and *Staphylococcus aureus* in 33.

During the War, attention was specially drawn by American workers to the occurrence, among troops under camp conditions, of epidemic broncho-pneumonia due to hæmolytic streptococci, and the disease was associated with a high carrier rate (see Irons and Marine, 1918; Levy and Alexander, 1918; and MacCallum, 1919). Most cases followed measles, but the condition was also the sequel of tonsillitis, diphtheria or bronchitis, while exposure and fatigue were important predisposing factors. The organism was apparently distributed, as a result of colds and bronchitis, from the nose and throat of sick persons and carriers. MacCallum concluded that hæmolytic streptococci, even without predisposing diseases, may give rise to extensive and fatal epidemics of a specific type of broncho-pneumonia which he designated 'interstitial'. The infection extended into the small bronchioles with an inflammatory condition of the air passages and further extension into the lymphatics round the bronchioles; later the pleura became involved with a resulting pleurisy and empyema. Secondary effects noted in some cases were mediastinitis, pericarditis, endocarditis, peritonitis, otitis, ulcerative laryngitis and septicæmia.

Hæmolytic streptococci are responsible for a proportion of cases of *empyema* occurring as a complication of pneumonia. One of the earliest references to the relative incidence of streptococci and pneumococci in empyema was by Netter in 1893. He showed that while the pneumococcus was more frequent in empyema of children, streptococci predominated in adults. Bythell (1903–4) also found that in children the pneumococcus was responsible for a large proportion of cases and streptococci were infrequent (about 5 per cent. of cases).

Scarlatina.

The ætiological relationship of streptococci to scarlatina was pointed out in 1887 by Klein, who regarded the disease as due to a specific type—'*S. scarlatinæ*'—which he found present in the throat and blood of cases. It was recognized, however, by later observers that the scarlatina

streptococci were similar to the pyogenic strains, that they produced, when inoculated into animals, no specific effects in any way resembling scarlatina, and that the persistent immunity following the disease contrasted with the transient immunity after recovery from other streptococcal infections. Though the association of hæmolytic streptococci with the angina of scarlatina had been fully established, until recent years the general tendency was to regard these as secondary invaders and not the primary causal agents (see Hektoen, 1907).

Bliss (1920, 1922) showed that hæmolytic streptococci could be isolated from the throat in all cases during the first week of the illness and then diminished in numbers, persisting for 10 to 20 days, and all recent observations have generally confirmed this.

Dochez and Sherman (1924[1 & 2]) claimed to have produced a scarlatina-like condition in animals by allowing streptococci of scarlatinal origin to grow in agar which had been introduced into the subcutaneous tissues. They suggested that a diffusible toxin is formed *in vivo*, which brings about the general effects of the disease. A further development of this work was the production of an antiserum from a horse immunized by the method used for experimental inoculation, and this serum injected intracutaneously ' blanched ' the rash of a scarlatina case, like the serum of a convalescent patient (see Blake, Trask and Lynch, 1924). The ' blanching ' phenomenon—the Schultz-Charlton reaction—produced by immune serum will be referred to later.

The first definite evidence of the ætiological relationship of hæmolytic streptococci to scarlatina was elicited by Dick and Dick (1923, 1924[2]), who produced the disease in volunteers by swabbing the throat with cultures of hæmolytic streptococci isolated from cases. Nicolle, Conseil and Durand (1926) have also produced scarlatina by tonsillar inoculation of a hæmolytic streptococcus isolated from the disease. Dick and Dick (1924[1]) showed that culture-filtrates of scarlatina streptococci, when injected into the skin, produce in susceptible persons a local erythematous reaction, i.e. the scarlatinal rash in miniature. In convalescents (i.e. immune persons) as a rule no such reaction occurs. This phenomenon, the ' Dick reaction ', which has been regarded as the analogue of the Schick reaction, has now been most extensively studied and its validity as an index of susceptibility to the disease on the one hand and of immunity on the other is generally accepted (Zingher, 1924[1] ; O'Brien and Okell, 1925 ; and others).

The reaction has been generally interpreted as due to a diffusible toxin formed by the organism *in vitro*, and identical with the toxic agent responsible for the general manifestation of scarlatina. Exceedingly minute amounts of a filtrate (e.g. 0·01 c.cm. of a 1 in 10,000 dilution) may yield definite reactions, and the subcutaneous injection of larger doses may produce a generalized scarlatiniform rash (Dick and Dick, 1924[2], and others).

Various methods have been applied in the preparation of toxin for

testing purposes. A potent filtrate (active in a dilution of 1 in 1,000) can be readily obtained by growing the organism for 5 days in bouillon (pH 7·5) containing 5 per cent. sterile defibrinated rabbit's blood. The culture is centrifuged and then filtered through a Berkefeld filter. Methods of purifying crude toxin preparations have been described by Huntoon (1924), Henry and Lewis with others (1925), Mackie and McLachlan (1926), Hartley (1928) and Pulvertaft (1928). According to Huntoon, the toxin is precipitated with the higher albumin fraction and is not a globulin. Hartley, following Walpole's method (1915) for the purification and concentration of diphtheria toxin, has succeeded in concentrating the scarlatina toxin 20- to 40-fold, removing at the same time 99 per cent. of the nitrogenous constituents of the crude material. This concentrated preparation can be reduced to dryness and retains its specific properties for at least 4 months. Toxin preparations can be kept in a 1 in 100 dilution, and diluted further when required. They retain their potency in dilution for long periods (see Joe and Swyer, 1928).

The amount of toxin injected in the Dick test is usually 0·1 to 0·2 c.cm. of a 1 in 1,000 dilution and the injection is made intracutaneously in the forearm. For control purposes a similar quantity of diluted toxin previously heated at 100° C. for 1 to 1½ hours is injected in the other forearm. The reaction appears in 6 to 12 hours as a bright red erythematous area varying in size but usually about 15 to 30 mm. in diameter. It attains its maximum in 24 hours and then gradually disappears. Occasionally pseudo-reactions result from the injection of heated toxin (see Zingher, 1924[2]). Different toxin preparations vary in their degree of activity ; the only satisfactory method available for standardizing these has been by cutaneous tests in susceptible subjects. Recent methods of producing a concentrated toxin lethal to laboratory animals offer a means of standardizing the toxin by a more exact method.

It has been assumed that the negative reaction is due to the presence of a neutralizing antitoxin in the blood. Scarlatina patients during the first five days of the disease as a rule exhibit a positive reaction while most convalescents are negative. According to Joe (1925), 95 per cent. of early cases give a positive result while from the thirty-first to thirty-fifth day only 4·7 per cent. are positive. Between these two periods there is a decrease in the percentage of positive reactions. Early cases obviously represent the susceptibles ; the convalescents are the immune. As shown by Zingher (1924[1]), young infants are frequently negative, and this is assumed to be due to a passive immunity derived from the immune mother. After six months this immunity is lost. The majority of adult persons react negatively. It has been supposed that immunity is acquired not only by passing through an attack of the disease but also by " sub-infections " which are not clinically recognizable. In this country, among fever hospital patients suffering from other conditions, Joe has found the highest incidence of positive reactions (60 per cent.) at 3 to 4 years ; in persons with a previous history of scarlatina 88 per cent. are negative.

It had been shown in 1918 by Schultz and Charlton that the intradermal injection of serum from convalescent patients blanches the rash locally in an early case. This extinction phenomenon was first interpreted by Mair (1923) as evidence of the specific neutralization *in vivo* of toxin by antitoxin from the recovered or immune person. A serum capable of blanching the scarlatina rash is also able to neutralize the artificially prepared ' toxin ' when the two are mixed and injected intracutaneously in a non-immune person. Further, the serum of ' Dick-negative ' persons usually gives the Schultz-Charlton reaction, while that of ' Dick-positive ' reactors fails to blanch the scarlatinal rash (see Joe, 1925). This would seem to indicate the identity of the specific toxin of scarlatina with that produced *in vitro* by the hæmolytic streptococci isolated from the disease.

It has also been found possible to immunize horses with the artificially prepared toxin, and their serum possesses definite antitoxic properties, neutralizing toxin in the Dick test and blanching the scarlatinal rash as in the Schultz-Charlton reaction. Such sera have come to be used in the treatment of the disease with some measure of success (Birkhaug, 1925[1] ; Blake and Trask, 1925 ; Dick and Dick, 1925 ; Park, 1925 ; and others). ' Dick-positive ' reactors may also be rendered ' negative ' within 48 hours by the injection of antitoxin.

Owing to the relative insusceptibility of animals to toxin preparations (vide *infra*) the usual method applied in standardizing this serum has been to inject mixtures of toxin and serum intracutaneously in Dick-positive subjects and in this way to estimate the neutralizing properties of the serum. Recent work on the production of concentrated toxins which are lethal to laboratory animals promises a new method of standardizing these antitoxic sera by means of an animal test. The official standard in the United States is the neutralization of 1,000 skin-test doses of toxin by 1 c.cm. of the serum. By means of a test involving the protection of rabbits from septicæmia produced by the intravenous injection of scarlatina streptococci, Parish and Okell (1927) have found it possible to distinguish efficient antisera, and in this way have been able to ' titrate ' antitoxin. The efficiency of an antitoxic serum can also be estimated by the ' blanching ' reaction.

' Dick-positive ' persons can be actively immunized with toxin and become in this way negative (Dick and Dick, 1924[3] ; Zingher, 1924[3] ; and others).

To sum up, the presence in the throat of large numbers of hæmolytic streptococci is a uniform feature of the disease at its earliest stages, and these organisms in culture produce a diffusible product which can be identified with the toxic agent responsible for the scarlatinoid syndrome.

Thus, in recent years scarlatina has come to be regarded as a primary infection by a hæmolytic streptococcus, the general effects being due to a specific exotoxin (Dochez, 1925 ; Okell and Parish, 1928) . ' Surgical scarlatina ' may be regarded as due to wound infection by hæmolytic streptococci producing the specific toxin. The same would apply to

puerperal scarlatina in which the toxigenic organism infects the uterus. On this basis also, immunity to scarlatina is considered as essentially an immunity to the scarlatina toxin and not necessarily to the invasion of the throat or other tissues by the particular streptococcus. Thus persons immune to scarlatina may still be susceptible to acute streptococcal angina without, of course, other symptoms or signs of scarlatina (Okell and Parish, 1928). Such cases must play a great part in spreading scarlatinal infection. Similarly, immune persons may become carriers of these streptococci without obvious clinical signs of either scarlatina or acute angina and distribute the infection.

The viability of '*S. scarlatinæ*' is of some epidemiological interest, and bacteriological observations on this subject are in accordance with clinical evidence regarding mediate infection in this disease. Balmain (1927) has shown that the organism may survive in experimentally contaminated books for 4 weeks, and von Jettmar (1927) has stated that it remains alive for 6 months in dried mucus from the throats of cases, and in cultures, without loss of toxigenic power. He states, further, that it is not destroyed by sunlight. We have also found cultures kept in the dark at room temperature viable after 6 to 7 months. The organism can survive, therefore, for considerable periods apart from the body.

Outbreaks of scarlatina which are apparently milk-borne, have been frequently recorded and generally supposed to be due to direct contamination of milk supplies with the specific virus from infected persons. This subject has recently been discussed by Jones and Little (1928), who instance natural infection of the cow's udder by scarlatina streptococci, associated with a resulting milk-borne epidemic of the disease. They have shown that in milk these organisms are inhibited from growing and are even killed, and regard the infection of the udder with the shedding of large numbers of streptococci in the milk as the explanation of milk-borne scarlatina. According to their findings direct contamination of milk from infected persons is a less likely factor in setting up such outbreaks. Streptococcal infection of the cow's udder (with resulting mastitis) derived from a human source and giving rise in turn to epidemic sore throat has been discussed in an earlier section (vide p. 84).

Though culture-filtrates of scarlatina streptococci are so highly toxic to the non-immune person, animals can tolerate even large doses. Intracutaneous injection usually produces no reaction in animals and the subcutaneous, intraperitoneal or intravenous injection of large doses has little or no effect (Dochez and Sherman, 1924[1 & 2]; Dick and Dick, 1924[5]; Rosenow, 1924; Okell and Parish, 1925). According to Kirkbride and Wheeler (1924, 1926), young goats are susceptible and yield a reaction to intracutaneous injection analogous to the Dick reaction. The relative insusceptibility of animals has introduced a difficulty in accepting the active principle of culture-filtrates as a true toxin analogous to the exotoxin of the diphtheria bacillus. Duval and Hibbard (1927) have found that a culture-lysate of *S. scarlatinæ* is ten-times more potent than the ordinary

filtrate, and have also elicited marked toxic effects in dogs by the injection of lysates, acute hæmorrhagic nephritis being a striking result. They have suggested, however, that the active principle of filtrates (' Dick toxin ') is of the same nature as that in lysates, i.e. an endotoxin and intracellular derivative of the bacterium. It seems difficult, however, to identify these two principles. Even large doses of filtrate are inert in animals, whereas most minute doses are highly toxic to the susceptible person. It might be assumed that filtrates contain a diffusible substance whose toxicity is highly specific for the human species. By means of purified and concentrated filtrates (Hartley, 1928 ; Pulvertaft, 1928) lethal effects have been produced in rabbits following intravenous injection. Hartley has succeeded in obtaining a concentrated toxin with an m.l.d. as low as 0·2 c.cm. It has also been shown that different types of rabbits vary in susceptibility, the Chinchilla rabbit being uniformly susceptible. It is noteworthy, however, that such concentrated toxin does not produce a cutaneous reaction in animals analogous to the Dick reaction (Pulvertaft). The question, of course, arises whether the principle in toxin preparations that gives the cutaneous reaction in the human subject is identical with that in the concentrated preparation responsible for lethal effects in animals.

Though the Dick reaction has appeared to be the analogue of the Schick reaction, the two phenomena show striking differences. The unconcentrated streptococcal ' toxin ' described above is highly thermostable as contrasted with diphtheria toxin and can only be inactivated by heating at 100° C. for 1 hour. The positive Dick reaction might seem to correspond with a cutaneous reaction of hypersensitiveness like the pseudo-reaction seen in testing for the Schick phenomenon. Further, as mentioned above, animals are relatively insusceptible to the specific toxin. It has also been suggested that the scarlatinal rash might be interpreted as an allergic phenomenon resulting from hypersensitiveness to the specific proteins of certain hæmolytic streptococci, the allergic state being brought about by previous subinfection with these organisms (Bristol, 1923). On this basis the Dick reaction might be regarded as an index of such sensitiveness and the negative reaction might be due to desensitization during the illness.

Though laboratory animals are normally insusceptible to unconcentrated filtrates of scarlatina streptococci, they can be sensitized by the injection of cultures or culture-filtrates so that the intracutaneous injection of the latter evokes a reaction analogous to the Dick reaction (Paraf, 1925 ; Dochez and Sherman, 1925). This effect was studied in guinea-pigs by Mackie and McLachlan (1927), who found that the reaction of cutaneous sensitiveness was not definitely analogous to the Dick reaction ; thus, high concentrations of filtrate were required to produce positive results which were rarely of marked degree, the active principle of the filtrate was not inactivated at 100° C. even after 3½ hours' heating, and the sensitive state was not specific ; filtrates from unrelated organisms produced reactions in the sensitized animals. Dochez and Stevens (1927) in sensitizing rabbits with an erysipelas strain of hæmolytic streptococcus

noted, however, different phases of sensitization ; at an early stage, a cutaneous reaction analogous to the Dick phenomenon could be elicited and the effect was annulled by injecting antitoxic serum along with the filtrate ; later, the sensitiveness became less specific like that noted by Mackie and McLachlan.

This question whether the scarlatina ' toxin ' is a true toxin or whether toxic effects merely manifest an allergic state requires further investigation. The production of specific antisera by immunization of animals with filtrates, the neutralizing effect of these sera and the lethal effects in animals produced by concentrated toxin (also annulled by antitoxin) would, of course, support the view that the active substance is a true toxin.

Certain further difficulties may be instanced in regard to recent theories of the ætiology of scarlatina ; a certain small percentage of convalescents remain ' Dick-positive ' ; Okell and Parish (1925) have stated that persons who have had scarlatina are as frequently ' Dick-positive ' as those who have never suffered from the disease. Various observers have also noted a high proportion of negative reactors among early cases (Silcock, 1925 ; Lees, 1927 ; and others). Lees has recorded cases which were negative prior to the illness. Ciuca, Balteanu and Thoma (1928), from observations of the Dick reaction in a large number of persons of different ages, have cast doubt on the validity of the test as an index of susceptibility or immunity to scarlatina. It is, of course, possible that other factors besides antitoxin contribute to immunity, and that the antitoxic immunity represented by a negative Dick reaction does not in all cases confer complete resistance.

It was at one time supposed that a filterable virus might be the specific infecting agent, the streptococcus being a secondary invader. Zlatogoroff, Derkatsch and Nasledyschewa (1926) have recently claimed that they have demonstrated a filterable virus in the disease, pathogenic to rabbits and monkeys, and have attempted to explain the known facts in the bacteriology of scarlatina by suggesting that this virus ' activates the streptococcus '. Their experimental data in favour of a specific virus are not conclusive.

Cantacuzène and Bonciu (1925) state that it is possible to produce a scarlatinoid syndrome in rabbits by injection of filtrates of pharyngeal secretion, urine or blood from cases of scarlatina during the febrile period. The blood-serum of animals that develop symptoms agglutinates scarlatina streptococci in high dilutions. The same workers (1926, 1927[1 & 2]) have also drawn attention to a peculiar phenomenon relating to the agglutinability of organisms by the sera of patients convalescent from scarlatina. If pharyngeal secretion or urine is taken from a case of scarlet fever during the acute stage of the illness, diluted with broth, and filtered through a Chamberland L3 filter, the filtrate has the power of rendering agglutinable, hæmolytic streptococci which are cultured in it, though obtained from sources other than scarlatina and not previously agglutinated by convalescents' sera. These claims have been supported by Martin and Lafaille (1926), and by Zoeller and Meerssemann (1927), and it has been found

that by a similar procedure other organisms besides hæmolytic streptococci may be rendered agglutinable by sera from convalescents. The principle responsible for this effect is used up quantitatively and is susceptible to extremes of temperature, being destroyed by exposure at 2° to 3° C. for 24 hours, or by heating at 60° C. for 2 hours. Its presence can be invariably demonstrated in early cases of scarlet fever and it disappears about the eighteenth to twenty-fifth day of the illness. A satisfactory explanation of this phenomenon has not yet been advanced.

Caronia and Sindoni (1923) and other Italian workers have described an anaerobic diplococcoid organism which they find in the disease and regard as the specific virus ; they have adduced experimental and immunological evidence in support of their view, but their findings have not received confirmation by workers in other countries.

An important part of the ætiological problem of scarlatina is the question whether the hæmolytic streptococcus associated with the disease is a specific type. Efforts to identify scarlatina streptococci by biological characters and biochemical reactions have been unsuccessful ; these strains do not in such respects differ from hæmolytic streptococci isolated from suppurative lesions and from simple inflammatory conditions of the throat (McLachlan, 1927). Earlier work suggested that scarlatina strains constitute a fairly well-defined serological group differentiated from others by agglutination reactions. Thus Bliss (1920) and Dochez (1925) claimed that 80 per cent. of scarlatina strains could be assigned to one group. Although the same standpoint was adopted by a number of subsequent workers (Bliss, 1922 ; Eagles, 1924 ; and others), it has now become certain that there are multiple serological types associated with scarlatina and that these cannot be distinguished serologically from hæmolytic streptococci derived from other sources (Griffith, 1926, 1927 ; Smith, 1927). McLachlan and Mackie (1928) in a further analysis of the serology of the hæmolytic streptococci found that while strains presenting certain serological characteristics are more frequently associated with scarlatina than with other lesions, hæmolytic streptococci with common serological properties may occur both in scarlatina and in various other conditions. No definite serological distinction could be drawn between strains from scarlatina and other sources, and it was impossible to define a ' *S. scarlatinæ* ' by serological methods. They also pointed out that there is so much overlapping in serological properties among the supposed groups as to make it doubtful whether the differentiation and relationship of strains can be expressed by formulating such ' serological groups ' or ' types '.

The question whether scarlatina strains can be differentiated by their specific toxigenic properties—as evidenced by the Dick reaction—has also been investigated by a number of workers. It has been found that culture-filtrates of strains isolated from erysipelas, puerperal fever and various other conditions may produce reactions similar to those yielded by filtrates of scarlatina streptococci (Rosenow, 1924 ; Williams, 1925 ; Birkhaug, 1926[2] ; Eagles, 1926). Rosenow (1926) has also stated that it

is possible by animal passage to stimulate a non-toxigenic strain to develop toxin. McLachlan (1927) has shown clearly that scarlatina strains resemble each other in their almost uniform power of producing a toxin active in high dilutions (e.g. 1 in 1,000) whereas a minority only of non-scarlatina streptococci produce toxin to an equal degree and these are mostly strains of throat origin. A number of non-scarlatina strains, however, yield toxic filtrates with properties similar to the toxin of the scarlatinal organisms but less active quantitatively. It was concluded that a certain proportion of hæmolytic streptococci from other sources possess a similar toxigenic power though only weakly developed. McLachlan also noted that the toxins of most of the scarlatina and non-scarlatina strains were neutralized by the antitoxic sera in use at the time for therapeutic purposes. A minority were not neutralized (see also Kirkbride and Wheeler, 1926). Such results suggest some variation in the antigenic characters of these toxins. Thus, if any difference exists between scarlatina and non-scarlatina strains it must depend on the more highly specialized toxigenic properties of the former; this, associated with a particular grade of invasiveness, may confer on strains an almost specific property of reproducing uniformly in passage among susceptible persons the scarlatinoid syndrome, so long regarded as almost a specific disease.

On the other hand it must also be recognized that such an infection in a person who is immune to the toxin may lead only to an acute infectious angina (Okell and Parish, 1928)—' scarlet fever *sine eruptione* '.

Infection of the Stomach, Intestine, Peritoneum.

The predominant streptococcal types found in the intestinal tract are non-hæmolytic in character, and hæmolytic varieties are of infrequent occurrence. According to Davis (1920), gastric juice of normal acidity kills hæmolytic streptococci in 2 to 5 minutes, and Moody and Irons (1923) have found that organisms of this type are destroyed by the juice in a dilution of 1 in 16 in 10 minutes. It would appear, moreover, that conditions in the alimentary tract do not favour their multiplication. Bile tends to inhibit their growth and sometimes exerts a bactericidal effect towards them (Stone, 1922). It has been the experience of most investigators that hæmolytic streptococci are infrequently encountered in the alimentary tract (Davis, 1919, 1920; Dible, 1921[2]; Davidson, 1928); on the other hand, organisms of this type may be more readily isolated from the alimentary tract in certain conditions, e.g. scarlatina, acute nephritis (see Moody and Irons, 1920; Wordley, 1922).

Enteritis and Phlegmonous Gastritis.

The role of hæmolytic streptococci as the cause of enteritis is uncertain and much of the older literature regarding the occurrence of streptococci in such conditions is difficult to analyse in the light of modern knowledge. Observations by Baermann and Eckersdorff (1909) would indicate that hæmolytic streptococci may cause a dysenteric enteritis, an organism of this type being present in considerable numbers in the stools of cases

investigated by these authors. Beck (1892) and Drasche (1894) have claimed that streptococci were the cause of cases of 'cholera nostras' investigated by them. A choleraic condition was produced in human volunteers following the ingestion of pure cultures of the organism isolated by Drasche.

It is difficult to assess the value of some of these early observations since it is well known that an increase in the numbers of intestinal streptococci occurs in bacillary dysentery, summer diarrhœa and other diarrhœal conditions.

Phlegmonous gastritis and enteritis are of rare occurrence: the presence of streptococci in these lesions has been reported by Sébillon (1885) and Askanazy (1895) respectively. The literature of the former condition has been collected by Robertson (1907).

Appendicitis.

Various types of bacteria have been found in appendicitis, but there is still doubt about the conditions which favour its occurrence and the ætiological importance of the various organisms associated with it. In the early stages the essential lesion is an interstitial inflammation of the wall with some ulceration of the mucous membrane. The early occurrence of ulceration is regarded as evidence against a hæmatogenous origin (see Muir, 1924; Warren, 1925). The bacterial flora reported by different investigators has varied greatly. The importance of non-hæmolytic streptococci and enterococci as the primary infecting agents has been emphasized by Rosenow (1915[1]) and Meyer (1928) respectively. According to Warren (1925) *B. coli* is the organism most frequently found, alone, or with other bacteria such as hæmolytic streptococci. Weinberg, Prévot, Davesne and Renard (1928) have found that streptococci are only occasionally encountered in cases of acute appendicitis, the organisms most commonly present being *B. coli*, *B. welchii* and the enterococcus. Haim (1907) considered streptococci to be the chief cause of appendicitis in children, while *B. coli* was more common in adults. Hæmolytic streptococci may be the ætiological agent in some cases. Kraft (1921) examined 77 pathological appendices and found hæmolytic streptococci in large numbers on four occasions, the lesion in these cases being of an ulcerative or gangrenous nature. The same observer was able to demonstrate hæmolytic streptococci in small numbers in 2 of 48 normal appendices. Dudgeon and Mitchiner (1923–4) and Meyer (1928) have also encountered hæmolytic streptococci on one occasion in their series of cases of acute appendicitis.

Peritonitis.

Though peritonitis is frequently caused by streptococci, alone, or in association with other bacteria, especially coliform bacilli, no distinction has, as a rule, been made in the different investigations between hæmolytic and non-hæmolytic varieties, and the frequency of occurrence of hæmolytic streptococci in this condition is therefore uncertain. Dudgeon and

Sargent (1905) carried out a bacteriological study of 270 cases of peritonitis, and concluded that *S. pyogenes* was rarely the ætiological agent. Fishbein (1912) collected the following data : of 342 cases investigated, coliform bacilli were found in 183, staphylococci in 83 and streptococci in 143. It is not stated what proportion of the streptococci were hæmolytic. Osler and Macrae (1920) refer to a series of 56 cases which followed inflammatory processes in the intestine ; in 43 of these coliform bacilli were found, usually in association with streptococci ; in 35 cases occurring after operation staphylococci were present alone in 12, streptococci in 5, and coliform bacilli in 5. In the female, infection may spread up the genital tract : this was apparently the mode of infection in 2 cases reported by Schwartz (1927) in which streptococcal peritonitis developed after vulvovaginitis.

Hæmatogenous peritonitis is frequently a terminal phenomenon, and streptococci are often the ætiological agents. Of 12 cases encountered by Flexner (1898) 5 were streptococcal. A very fatal streptococcal peritonitis of hæmatogenous origin has also been observed in young children of both sexes by Lipshutz and Lowenburg (1926) and Schwartz (1927). The majority of the cases reported by the former authors were due to hæmolytic streptococci. Schwartz has reviewed the literature dealing with this condition, and concludes that there is a preceding infection of the throat in the majority of instances.

Infection of the Genito-urinary Tract.

Though many of the factors concerned in the production of nephritis are obscure, there is considerable evidence that micro-organisms and their toxins are frequently of ætiological importance. While non-hæmolytic streptococci may in some cases be the causal agents, acute nephritis may also develop in the course of acute pyogenic processes, including infections with hæmolytic streptococci, e.g., Ludwig's angina, puerperal septicæmia, etc. (Dunn and Thompson, 1921 ; Gray, 1928). The occurrence of nephritis in scarlatina is well known and may be interpreted as a toxic phenomenon. In the causation of non-suppurative nephritis the relative importance of bacterial toxins and of an actual invasion of the kidney by micro-organisms is still undetermined. In many cases no bacteria are demonstrable in the kidneys and the lesions are regarded as of toxic origin.

Acute suppurative nephritis may follow septic endocarditis, wound infections, scarlatina and puerperal fever, and may be part of a general pyæmia.

Infections in other parts of the genito-urinary tract are generally due to organisms other than hæmolytic streptococci, and where streptococci are described in the literature little information is given regarding their exact classification.

Streptococci may be associated with secondary infections of the urethra and adnexa after gonorrhœa, but definite information is wanting in

regard to the exact biological types ; from the morphological description it would appear that the majority of them may be enterococci. Thomson (1923) has summarized the bacteriological investigations on this subject. Primary streptococcal urethritis may occur and Luys (1922) cites a case in which contact infection apparently resulted from a case of erysipelas. Culver (1916) has cultivated hæmolytic streptococci from persons with chronic prostatitis and spermatocystitis, sequelæ of an earlier gonococcal infection.

Puerperal Infection.

The part played by streptococci (both hæmolytic and non-hæmolytic types) in puerperal infection has been well established. Various pathological conditions are included under the general designation of puerperal infection : inflammation and suppuration of a perineal wound, vaginitis, endometritis, metritis, parametritis, salpingitis, oöphoritis, peritonitis, phlebitis, phlegmasia, pyæmia and septicæmia. The commonest lesion is endometritis. After delivery the placental site is analogous to an open wound and the thrombosed sinuses communicate directly with the uterine cavity. The conditions existing afford invasive bacteria an easy entrance into the tissues. Thus the endometrium may become infected, or the venous thrombi are invaded and septic phlebitis results. The consequences of such infection must depend primarily on the virulence of the organisms present ; and a highly invasive streptococcus or other organism may spread in the lymphatics, producing metritis, parametritis or peritonitis, or by the veins, leading to septicæmia or pyæmia. The uterine infection may be entirely localized but nevertheless may lead to a severe inflammatory lesion. In some cases the inflammatory condition spreads into the Fallopian tubes either by the natural channel or by lymphatics ; the ovary may also be involved, oöphoritis being usually associated with parametritis. The infection of thrombi and the setting free in the veins of septic emboli brings about a pyæmic condition. Spreading thrombosis may involve the internal iliac veins and extend to the common iliac vessels, the vena cava and the external iliac veins (resulting in the condition of phlegmasia). According to Halban and Köhler (1919) the starting-point of the infection may be a perineal wound, a tear of the vagina or cervix, but is most frequently the interior of the uterus. Spread takes place by lymphatics, the blood-vessels or along the tubes to the peritoneum. In blood infections, metastases occur in the skin, lungs, pleuræ and periarticular tissue, and endocarditis may also result. Werner and Zubrzycki (1914) in 200 cases of puerperal infection found streptococci in 182 (102 in pure culture) ; of these, 118 were hæmolytic strains : their fatal cases were all streptococcal. Halban and Köhler (1919) in a series of cases of blood infection found streptococci to be the commonest organism, the majority of these being of the hæmolytic type (56) ; non-hæmolytic strains were less frequent (24). Similar results to these have been obtained by various other workers.

Puerperal sepsis has generally been regarded as an exogenous infection brought about by the introduction of virulent pyogenic organisms into the genital tract at the time of labour by the hands of attendants, instruments, &c., and there can be little doubt that contact infection plays a frequent part in the ætiology of the condition. Evidence of the transmission of the disease from one puerperal case to another by medical and nursing attendants is beyond question. Furthermore, it has been shown that pyogenic organisms may be transmitted to puerpera from other inflammatory or suppurative conditions due to hæmolytic streptococci, e.g. erysipelas, scarlatina. Reference has already been made (p. 90) to the occurrence of the so-called puerperal scarlatina.

The possibility of pyogenic organisms present in the vagina at the time of labour producing a uterine infection also requires consideration. Thus, it has been recognized that as a result of manual examination or manipulation or instrumentation, &c., bacteria from the vulva or vagina may be introduced into the uterine cavity, and that an endogenous infection may result in this way. Bumm and Sigwart (1912) found streptococci in the vagina in 69 per cent. of pregnant women and in 72 per cent. of cases of labour; of these, 15 per cent. were hæmolytic strains, but their presence did not apparently lead to uterine infection. Schäfer (1917) reported similar results; cases with hæmolytic or non-hæmolytic streptococci in the vagina before labour passed through apparently normal puerperia. Bigger and Fitzgibbon (1925) have shown that streptococci occur in the vagina before and after delivery in 68 per cent. of cases—mostly *S. fæcalis* and *S. mitis* types. Thus potentially pathogenic streptococci may be present in the genital tract before labour without necessarily giving rise to consequent infection of the uterus; on the other hand the possibility of auto-infection may reasonably be postulated. The occurrence of such infection must depend on the virulence of the strains present and the general or local immunity of the individual; thus severe hæmorrhage, exhaustion, and instrumental or manipulative trauma would undoubtedly play a part in promoting infection by pyogenic organisms present in the genital tract. Bigger and Fitzgibbon have suggested that an infection due to a hæmolytic streptococcus is probably exogenous, while that due to non-hæmolytic types is endogenous.

It must also be recognized that puerperal infection due to hæmolytic streptococci may emanate from attendants who at the time are carrying virulent strains in the throat and nose (Meleney, Zaytzeff, Harvey and Zau, 1928).

The hæmolytic streptococci from puerperal infections comprise many strains with toxigenic properties similar to those from scarlatina (Eagles, 1926; Lash and Kaplan, 1926; and others, see also p. 93); further, the toxins of such organisms are neutralized by a scarlatina antitoxic serum. It seems doubtful whether the toxin of puerperal strains differs essentially from that of scarlatina streptococci.

Cutaneous reactions with toxins from scarlatina and puerperal strains have been investigated (on the analogy of the Dick reaction) with a view to ascertaining whether any relationship exists between sensitiveness to toxin and the occurrence of infection. According to Lash and Kaplan, women shortly after labour yield a higher proportion of positive reactions with toxin from puerperal streptococci than pregnant women. This finding would, of course, suggest an increased susceptibility to the streptococcal toxin in the puerperium. Burt-White (1928) states that women who react to scarlatina toxin are more liable to develop sepsis than non-reactors. These reactions require further investigation as regards their relationship to the occurrence of puerperal infection. It has been shown (p. 92) that immunity to toxin is no index of resistance to the organism itself.

According to Eagles (1926) the puerperal strains of hæmolytic streptococci form a distinct serological group (see also Lash and Kaplan, 1925) ; the specific serological grouping of the scarlatina, erysipelas and puerperal streptococci respectively cannot, however, be accepted (vide p. 93). Thus, puerperal infection, localized or general, is usually due to streptococci, the common type being hæmolytic, though non-hæmolytic strains may produce serous lesions and even septicæmia. While auto-infection may occur under certain conditions, it must be assumed that most cases are the result of exogenous infection with virulent strains transmitted from pre-existing cases, from other streptococcal conditions such as erysipelas, scarlatina, streptococcal sore throat, and in fact any septic lesion. In addition, carriers of virulent organisms (e.g. in the throat) among patients of lying-in hospitals, medical attendants and nurses may originate sporadic cases and even groups of cases.

The Mouth Streptococci.

By H. D. Wright.

The streptococci considered in this section possess certain characteristics common to the genus. They have, however, some peculiarities which enable them to be differentiated from the true hæmolytic and the fæcal streptococci. Unfortunately they are so heterogeneous as a group that it is difficult to apply to them any sufficiently comprehensive name. Probably the term *Streptococcus viridans* (Schottmüller, 1903) most nearly fulfils the requirements of the situation, expressing as it does a form of activity common to the great majority of the organisms, but it excludes some which strictly belong to the group. They are most characteristically found as saprophytes upon the mucous membranes of the upper part of the alimentary canal and the respiratory passages of man. Gordon (1905[2]) found them to be the best indicator of the amount of salivary contamination of an atmosphere. Although some of the older writers found them in the mouths of only about one fourth of normal people, they can be demonstrated in practically all mouths in the entire absence

of any recognizable pathological process. Similar organisms have been isolated from the intestinal contents of man in small numbers (Dible, 1921[2]), and from the vaginal secretions of healthy women. One member of the group, found most commonly in horse dung, was also demonstrated by Andrewes and Horder (1906) to be frequently present in London air.

Streptococcus mucosus, for long regarded as a streptococcus, has been found to be more properly considered as a pneumococcus (Park and Williams, 1905 ; Levy, 1907). The very great majority of the strains are bile-soluble and pathogenic for mice. Serologically they fall into the Type III of the American workers. It is to be noted, however, that Dochez and Gillespie (1913) found one hæmolytic and bile-insoluble strain amongst nine which in their other characters, obvious capsulation and large mucoid colony, corresponded to *Streptococcus mucosus* as originally described by Schottmüller.

Morphology.

The individual organisms possess the general characters of the streptococcal group. They occur in chains which tend to be longer than those of *S. fæcalis*, and rather shorter than those of the hæmolytic streptococci. This character is, however, very variable, depending upon the medium used and upon the peculiarities of the individual culture. Freshly isolated strains, particularly those obtained from the blood of cases of infective endocarditis, tend to form longer chains than do subcultures of the same organism. Within the chains the organisms may occur singly or in pairs, the individual members being either spherical or slightly elongated. Variation in size is not uncommon. Much has been written tending to suggest that pleomorphism is characteristic of the streptococci found in association with certain diseases, but Norton, Rogers and Georgieff (1921) have pointed out that this is not the case.

Capsulation does not occur.

Cultivation.

In their growth upon solid media these streptococci resemble most closely the pneumococci. They develop in small discrete colonies which show little tendency to coalesce. The growth is much less luxuriant than that of *S. fæcalis* and much less confluent ; that of *S. hæmolyticus* is also usually more vigorous. Growth upon gelatin at 20° C. is uncertain, whereas the fæcal and hæmolytic organisms usually grow well (Andrewes and Horder, 1906). Media prepared from commercial meat extracts are not very suitable for the cultivation of these streptococci. Preparations from fresh infusions are much to be preferred, and enrichment with serum or some other body fluid is advisable. The best solid medium is undoubtedly some form of blood-agar.

On such media the colonies are small and variable in appearance, tending to be circular and elevated. In some cases they can only be detached as a whole from the medium. In 24-hour cultures they may resemble pneumococcal colonies very closely, but after 48 hours the latter

tend to show a central depression, expressive of autolysis, which is absent from the streptococcal colonies. Crowe (1923, 1924) has drawn attention to certain types of colony formed upon heated blood-agar, which he considers useful aids to the recognition of different types of streptococci. These characters are, however, not maintained in subculture.

In liquid media freshly isolated strains usually form deposits, but after a few days of repeated daily subculture the growths become much more evenly dispersed through the medium. The fæcal streptococci are evenly dispersed from the beginning ; the hæmolytic streptococci have a much greater tendency to form deposits. Serum or blood enrichment greatly facilitates growth and may be necessary with some strains. The reaction of liquid media should be in the neighbourhood of pH 7·5 to 7·8. Simple peptone solutions do not give satisfactory growths of some strains, and are, therefore, not a very suitable basis for the testing of fermentation activities. Hiss's serum-water medium is also unsuitable for some cultures. A peptone solution (1 per cent.) adjusted to pH 7·6, to which is added about 5 per cent. of rabbit- or horse-serum, provides a suitable basis for such tests.

For the initial cultivation of these organisms from secretions or exudates in which they may be present in small numbers a broth made from fresh infusion of veal, buffered with 0·2 per cent. of di-sodium phosphate, containing 0·2 per cent. of glucose, and enriched with 10 per cent. of serum is well adapted. For isolation from mixtures with other organisms the telluric acid medium described by J. F. Smith (1914) seems worthy of further investigation. Rosenow (1915[1]) has maintained, and in subsequent publications has further insisted on, the fact that many of these organisms do not grow in the presence of large concentrations of free oxygen, but do so at lower oxygen tensions. This work does not appear to have received adequate confirmation. The writer's experience with the organisms causing endocarditis (Wright, 1925) and with a large number of strains from the normal vagina suggests that the great majority grow freely under both aerobic and anaerobic conditions, but some appear to grow better anaerobically.

The maintenance of these streptococci in subculture is always a matter of some difficulty. Each individual strain requires to be studied in this connection. Frequent subculture is necessary and rich media must be employed—blood-agar, blood-broth or serum-broth. Serum-broth inoculated heavily and preserved without previous incubation in the refrigerator has proved useful for some strains. Pulvermacher (1922) points out that methods which are useful for preserving hæmolytic streptococci are not suitable for the ' green streptococci ', and Dible found the fæcal streptococci to be very hardy. In this respect this group of streptococci resembles pneumococci although they do not readily autolyse. The probable explanation is that the organisms produce a toxic peroxide, possibly hydrogen peroxide, with which they are unable to deal owing to the fact that they are poorly supplied with catalase (McLeod and Gordon, 1922[2]).

The changes produced in media which contain blood have been considered at length in discussing the streptococcal group as a whole, and it need only be stated here that the most frequent mouth streptococcus produces green discoloration on blood-agar whether the blood is fresh or heated, and corresponds to the *viridans* type of Schottmüller and the α type of Brown. The group, however, includes other forms which do not produce green discoloration in blood-agar media.

Fermentation Reactions.

Gordon (1905[2]) found that of 300 streptococci from saliva 211 fell into a certain grouping, to which the name *S. salivarius* was subsequently applied by Andrewes and Horder (1906). Its main feature was that the organisms failed to ferment mannitol while some of them attacked raffinose. Andrewes and Horder, extending the application of Gordon's tests, describe three main groups among the streptococci under discussion in accordance with Table III.

Table III.

Type	Growth on gelatin at 20° C.	Lact.	Man.	Sal.	Sac.	Raf.	Inulin	Coniferin	Milk	Neutral red reduction
Equinus ..		−	−	+	+	−	−	+	−	−
Mitis ..		+	−	+	+	−	−	+	−	±
Salivarius..	±	+	−	−	+	+	−	−	AC	+

− = no fermentation. AC = acid and clot.
+ = fermentation. ± = irregular results.

These were regarded as type reactions, of which many variant forms were discovered. Buerger (1907), Gordon (1910), Broadhurst (1913, 1915) and Thro (1914), all emphasized the fact that the quality of the basal medium has a great effect on the consistency of the results obtained. This is of particular importance in relation to the delicate organisms classed under the heading *S. viridans*. As mentioned above, a peptone medium containing about 5 per cent. of serum and adjusted to the optimum reaction (pH 7·6) is suitable for most strains, and on such a medium the results obtained are very constant. Certain strains, however, which are more delicate and not vigorous fermenters of the carbohydrates, show slight irregularities. This finding is in general agreement with most recent work on the subject (Holman, 1916[1]; Brown, 1919). Holman's classification extends the list of names in the group, and Brown's takes into account the various types of hæmolysis described above. The former has come to be used very commonly in current literature and the reactions of the non-hæmolytic streptococci are given in Table II (p. 37).

In addition, raffinose- and inulin-fermenting varieties are recognized. There is general agreement that mannitol fermentation is characteristic of fæcal streptococci while its absence is the rule for the mouth streptococci. Holman, for example, states that of the organisms from the nose and throat 127 were *S. mitis;* 155, *S. salivarius;* 9, *S. equinus;* and 6, *S. fæcalis*. On the other hand, organisms similar to the mouth streptococci may be isolated from the intestine (Dible, 1921[2]) and from the vagina (Bigger and Fitzgibbon, 1925[2]). It is not possible to differentiate streptococci from pneumococci by fermentation tests. Inulin fermentation is not at all uncommon among the mouth streptococci.

Any system of classification of streptococci based on carbohydrate fermentation is naturally dependent on the substances selected. It is to a large extent artificial as Andrewes and Horder say, like taking a base line at a certain height so as to make a series of mountain peaks look distinct : the higher the level chosen the fewer will be the peaks, or species. But the multiplicity of types probably does, as they also suggest, fairly represent a reality in the heterogeneousness of the group. This is to a certain extent substantiated by the fact that other methods of classification come to the same result and classifications by different methods do not coincide. At the same time a certain descriptive value may be attached to such a classification.

Heat Resistance.

These streptococci are readily destroyed by heating to 60° C. Dible (1921[2]) found that enterococci resisted this temperature for at least half an hour, whereas mouth streptococci and similar organisms isolated from the fæces did not do so. Similar results have been reported by Bagger (1926).

Effect of Bile.

Neufeld discovered that pneumococci were readily dissolved by bile whereas other organisms were not. Mandelbaum (1907) applied this test to streptococci and observed that in a concentration of 5 per cent. bile-salts kill streptococci but do not dissolve them. Upon this test rests the main method of differentiation between pneumococci and streptococci. It will be discussed further below (p. 110).

Weissenbach (1918) incorporated 10 per cent. of ox-bile in broth medium and found that this inhibited the growth of non-hæmolytic and of hæmolytic streptococci but not of the enterococcus. Stone (1922) observed that rabbit-bile (and no other) failed to inhibit the growth of mannitol-fermenting cocci whether hæmolytic or not and of some others. Bagger's studies (1926) indicate that pure ox-bile serves as a differential medium for distinguishing between enterococci and other streptococci, the former growing indefinitely in it while the latter fail to grow.

Summary of Distinguishing Features.

The mouth streptococci then will be seen from the biological point of view to be a heterogeneous group of organisms which have, however, in

common certain characteristics permitting of their differentiation from the other streptococci and the pneumococci, their neighbours on either hand.

These are summed up in Table IV.

TABLE IV.

	Hæmolytic Streptococci	Enterococci	Mouth Streptococci	Pneumococci
Chain formation.. ..	Long	Short	Moderate	Diplococcal and short.
Capsulation	—	—	—	+
Maintenance in culture ..	Relatively easy.	Very easy	Difficult	Difficult
Peroxide production ..	+	—	++	+++
Hæmolysis on blood-agar	β	γ	α or γ	α
Hæmolysis of suspension in saline.	+	—	—	+ after autolysis.
Mannitol fermentation..	Chiefly —	+	—	—
Heat resistance (60° for 30 minutes).	—	+	—	—
Bile-solubility	—	—	—	+
Inhibition of growth by bile.	+	—	+	+
Mouse virulence.. ..	Variable	Low	Low	High

Reliance upon any one of these tests may lead to error, but if the sum of the results be taken into account any given organism is not likely to be placed in the wrong group, and few will give rise to difficulty.

SEROLOGICAL CLASSIFICATION.

The study of the serological reactions of the streptococci in general is beset with difficulties owing to the relative instability of the bacterial suspensions. This difficulty is not so great in the group of mouth streptococci. Cultures upon solid media are usually less satisfactory than those in liquids. In the latter newly isolated strains may be unstable, but repeated daily subculture of small amounts of the supernatant fluid in a broth of pH 7·6 will in the great majority of cases, after about a week, yield a culture which is quite sufficiently stable for agglutination tests by the macroscopic method. Rarely a strain will be stable at room temperature (15 to 16° C.), but will flocculate in saline when heated to 55° C.

The sera of animals immunized against these organisms usually contain quite considerable amounts of the ordinary antibacterial antibodies, agglutinins, precipitins, opsonins and complement-fixing substances. Bactericidal action is slight or absent. The response of rabbits to intravenous injections is relatively good, though individual strains and

individual animals may be less satisfactory. According to Kinsella (1918), they are more efficient stimulants to antibody production than the hæmolytic streptococci. Too rapid increase of dosage in the early stages of treatment may lead to wasting and death of the animals. If living organisms be used in the later stages endocarditis or arthritis sometimes occurs. In horses the response is slow and the agglutinin titre of the serum not very high. The writer has observed the development of agglutinins in one man as a result of subcutaneous injections of rather large doses of vaccine (up to 1,000 million organisms).

Stable suspensions of these streptococci are apparently not agglutinated by normal human sera, or normal rabbit sera. On the other hand, agglutination by the serum of normal horses in low dilutions occurs not infrequently.

There is some slight disagreement in the reports of attempts made to classify the streptococci by serological methods. Kligler (1915) found that agglutination did not tend to separate the streptococci into large groups. Krumwiede and Valentine (1916) found the strains from pathological sources heterogeneous, but rather more tendency to grouping among the strains from the tonsils. Gordon (1922) and Norton (1923), using agglutinin absorption as the criterion, agree in finding them to be heterogeneous.

Kinsella and Swift (1917) employed the complement-fixation test. Using an antiformin solution of bacteria for this test against the sera of animals inoculated with whole bacteria they first demonstrated a great degree of homogeneity amongst the hæmolytic streptococci. On the other hand, the ' non-hæmolytic ' streptococci (corresponding largely to the group under discussion) showed considerable heterogeneity. They thought they were justified in concluding that the organisms on the whole tended to divide themselves up into two main groups which they term ' right- ' and ' left-hand ' groups from the position they occupy in their tables. Later (1918), Kinsella claimed to have demonstrated a certain relationship, still rather indefinite, between the hæmolytic streptococci and the ' left-hand ' group of ' non-hæmolytic ' streptococci. Howell (1918), using whole bacteria for the complement-fixation test, could get no similar grouping amongst the ' non-hæmolytic ' streptococci. Hitchcock (1924[1]) employed sera prepared by injections of whole bacteria, and tested them against a preparation of the products of streptococci which contained little or no protein—a ' residue antigen ' in Zinsser's sense—and observed that while preparations from streptococci of the hæmolytic group reacted identically in giving precipitates with all sera similar preparations from the ' non-hæmolytic ' streptococci did not so react. In other experiments (Hitchcock, 1924[2]) he found that sera prepared against whole bacteria of the latter group contained precipitins which showed great specificity when tested against solutions of a certain fraction of these organisms. They did not react with preparations of the hæmolytic streptococci. He concludes that any relationship which exists between the two groups

of streptococci is dependent not on the specific precipitable substance but probably on the protein or protein lipoid fraction. Later (Hitchcock, 1925), using another type of soluble product for complement-fixation tests, he, to a certain extent, confirmed Kinsella and Swift's work, finding that the ' left-hand group ' showed some relationship with the hæmolytic streptococci, while the ' right-hand ' group was apparently related to the pneumococci. This somewhat confused state of affairs was considerably clarified by the work of Lancefield (1925[1 & 2]). She applied the methods devised by Avery and Heidelberger for studying the antigenic structure of the pneumococcus. Sera prepared against the protein fraction were found to precipitate the proteins derived from other ' green ' streptococci, from hæmolytic streptococci, pneumococci and to a certain extent staphylococci. They did not react with derivatives of *B. coli* or *C. diphtheriæ*.

With the aid of absorption tests Lancefield was able to determine that the protein fraction of the non-hæmolytic streptococci consisted of three fractions. One of these was common to all such organisms and peculiar to them; a second was also found in hæmolytic streptococci and a third in all streptococci and also in staphylococci. Some evidence indicated that the protein of the individual strains differed slightly. The precise relation to the pneumococcal protein was undetermined. The non-protein and possibly polysaccharide substance in each group is strictly specific. It is not antigenic in itself (does not stimulate antibody production) when dissociated from the other constituents of the bacterium. When, however, the whole bacterium is injected it stimulates the production of antibodies which react with the separated carbohydrate to produce a typical precipitate of the same compact kind as in the case of the pneumococcus. Sera prepared against whole bacteria contain a predominant amount of specific anticarbohydrate substance and a varying amount of antiprotein substance which gives rise to the cross reactions with members of other groups. On this basis a great deal of the confusion in reported results can be referred to differences in details of technique. This work confirms the view that while there is a certain degree of relationship the organisms yet show a considerable degree of individuality. Serologically, as biologically, the group is heterogeneous, but classifications by the two methods have, in most workers' hands, failed to agree. This does not seem very surprising in a group of essentially ' wild ' organisms living a more or less saprophytic existence on the mucous membranes of the animal body.

Pathogenicity.

The pathogenicity of the organisms for experimental animals is low. The great majority of strains fail to kill mice when injected in quantities of 1 c.cm. of broth culture subcutaneously or intraperitoneally. Even much larger doses may be without apparent effect. This is in marked contradistinction to the results obtained with pneumococci, the majority of which are highly pathogenic for mice. Some authors suggest that the

strains obtained from cases of endocarditis are more virulent than other strains, but the difference, if present at all, is very slight. Guinea-pigs are similarly refractory. In rabbits, whatever the source of the streptococci, intravenous injections of relatively large doses of culture give rise to lesions involving the joints and the endocardium. The former are as a rule non-suppurative and associated with sterile effusions into the joint cavity. They may pass on into a subacute or chronic condition. The endocarditis is of a malignant type. These and other effects will be discussed further in connection with endocarditis and rheumatism. Subcutaneous injections are usually without effect. Lesions similar to those occurring in rabbits have been produced in small monkeys.

In agreement with the lower virulence of these streptococci, Lehmann (1926[1]) has observed that while hæmolytic streptococci grow readily in human defibrinated blood, the ' non-hæmolytic ' streptococci are either destroyed completely or grow very slowly. Schottmüller (1924) has reported similar observations.

Birkhaug (1927[1]) has recently reported that he has been able to obtain evidence of the existence of toxins in filtrates from cultures of some ' green producing ' and some ' anhæmolytic ' streptococci isolated from the throats of persons suffering from acute rheumatism. The effect of these is to produce local reactions on injection into the human skin in susceptible persons. This statement awaits confirmation.

Mutation.

Purely morphological considerations have from early times suggested that streptococci and pneumococci are closely related organisms and similarly that the various types of streptococci are very intimately connected together. The natural tendency is to look upon the semi-saprophytic organisms of the mouth as providing a matrix out of which have evolved certain members better adapted to a parasitic life within the host, a more dangerous, pathogenic group comprising in particular the hæmolytic streptococci and the pneumococci. This is the view suggested by Aschner (1917) and by Andrewes (1906). Admitting the minor variations which may occur in the matter of fermentation reactions and morphology, the main question at issue is whether the criteria generally adopted as separating the streptococci of the group here discussed from the hæmolytic streptococci on the one hand and from the pneumococci on the other are sufficiently constant to be relied upon.

Mutations affecting Hæmolytic Properties of Streptococci.

In the long and much-documented polemic upon the mode of infection in puerperal febrile conditions there has always been a school which maintained that hæmolytic activity is an inconstant and variable property, readily laid aside and equally readily resumed or assumed when the conditions are appropriate. Zangemeister (1910), for instance, draws attention to the change in the streptococcal flora from non-hæmolytic to

hæmolytic when inflammatory processes develop in the mucous membranes, and appears to regard this as a mutation affecting the organisms of the normal flora which individually change and then as the inflammation subsides recover their ordinary functions. Argument of this type is, of course, quite unconvincing. It might equally well be applied to the change in the intestinal flora in typhoid fever. McLeod (1912) has referred at some length to much of this earlier work. The greater part of it is concerned with showing that certain strains of hæmolytic and non-hæmolytic streptococci lose or gain hæmolytic power on continued culture on blood-agar or other media. McLeod and Brown (1919) have both stated that changes of this sort are extremely rare occurrences, and with this finding most experienced workers seem to be in agreement. The question was studied experimentally by Rosenow (1914[2&4]) working in many cases with cultures from single cells. He states that he was able by alterations in oxygen tension, by growing organisms ' in symbiosis with ' *B. subtilis* and by varying the salt-content of the medium to bring about all kinds of variations at will. There does not appear to be any independent confirmation of this work.

Some authors (Freund and Berger, 1924) have reported that the organisms isolated from the blood of cases of endocarditis vary in their hæmolytic activity at various stages of the disease, the variation being considered a consequence of variations in the resistance of the patient. Schottmüller (1924) has not been able to observe this, and the writer has similarly failed. It is not very uncommon to find a hæmolytic coccus invading the blood-stream just before death, but it is usually easy to determine that it differs from the organism causing the endocarditis in other respects than its action on blood-cells. In one case an apparent instance of this type of variation did occur. It was soon found, however, to be due to the fact that the organism did not produce hæmolysis of a suspension of red corpuscles and tended to throw off less hæmolytic colonies on the blood-agar plate. These variants again produced hæmolytic and non-hæmolytic colonies on subculture. No hæmolysis was ever produced in a liquid medium and the organism probably belonged to Brown's α-prime group.

Kuczynski and Wolff (1920, 1921[1&2]) have described experiments wherein they have injected hæmolytic streptococci into mice, and on examining the organs within a short time, 30 minutes to 3 hours being the most favourable interval, have succeeded in isolating some green-producing strains, the lungs being the organs in which they most commonly found the variants. This could be done only with certain strains and the change was not observed on artificial media. Immunized mice were more suitable for this experiment and the alteration could be effected in plasma-leucocyte mixtures derived from resistant, but not in those derived from susceptible, animals. ' *Viridans* sepsis ' on this view is not due to a special type of streptococcus but is an infection with hæmolytic streptococci occurring in a resistant animal. The green variants lost their virulence for mice

and might breed true in subculture, while others (' *pseudo viridans* ') recovered their hæmolytic property. Some on injection into mice might increase in virulence and come to be indistinguishable from pneumococci. Schnitzer and Munter (1921[1 & 2], 1923) observed similar phenomena. They stress the importance of using a sublethal dose. Work on similar lines is reported by Fréund (1923), Schnitzer and Pulvermacher (1923) and Hubert (1925). These workers appear to regard the change as induced by the animal fluids and not due to selection of certain variants in the culture. At the same time they all insist that it occurs very rapidly and after a few hours at most cannot be observed, only hæmolytic strains being isolated. It is extremely doubtful if any multiplication of organisms can occur in the mouse within the time stated. Moreover, it must be noted that the organisms employed are not sufficiently defined to permit of any valid conclusions from the experiment. In this type of work it is essential that the organisms used and the organisms recovered should be tested in every possible way. Kuczynski and Wolff state they take into account Brown's work on the types of hæmolysis, but none of these authors use any of the recognized methods of testing the identity of their strains. Experimental work upon variation involving the use of animals, particularly mice, is exposed to serious error and unless extremely carefully controlled is practically valueless. None of these authors report any control experiments upon the organs of normal mice.

Howell (1924) attempted to repeat these experiments and did succeed in isolating a few green-producing variants in a small number of animals. The percentage was not higher than in cultures, and any green-producing strains which were recovered produced hæmolysis in subculture. Lehmann (1926[1]) obtained similar results with 34 out of 37 strains. The remaining three showed certain variations when grown in milk and similar variation when injected into mice. He points out that the test of resistance to rivanol, which is sometimes relied on to differentiate hæmolytic and ' green-producing ' strains, is subject to wide variations. Reimann (1927[1]) failed to produce change in hæmolytic streptococci by exposing them to rivanol. Krumwiede and Valentine (1922) record what may well be a change from hæmolytic to ' green-producing ' without antigenic modification. This appears to have occurred spontaneously.

It would appear justifiable to conclude that mutation of this type does not readily occur, and that the evidence suggesting that it is frequent is inconclusive, chiefly because sufficient attention has not been paid to the different types of hæmolysis that may occur, or to the identification of the organisms which have been used. To base any theories of infection upon these experiments does not appear to be warranted.

Mutation from Pneumococcus to Streptococcus.

Morgenroth, Schnitzer and Berger (1925) state that by exposure of pneumococci to optochin suspended on yeast the organism becomes modified in a certain way (modification A), so that when subsequently

treated with an appropriate concentration of the drug a further change (modification B) occurred. This derivative was bile-insoluble and was non-pathogenic and so was indistinguishable from a green-producing streptococcus. Occasionally when this organism was injected into mice it became a hæmolytic streptococcus. Changes in the reverse direction also occurred. Similar changes in the course of passage experiments are recorded by Berger and Engelmann (1925).

Reimann (1927[1]) has repeated this work and has found that in weak solutions of optochin (1 in 600,000), and also in the presence of yeast suspensions, with or without adsorption of optochin, pneumococci undergo a change similar to what he had observed in the presence of bile solutions and immune sera (1925) and in capsules placed in the bodies of normal and immune animals (1927[2]). These changes consist in a loss of the substance conferring upon them their specific serological characters. The organisms become as it were masses of naked nucleoprotein reacting with serum prepared against any pneumococcus or against streptococci, which, as indicated above, contain the same type of nucleoprotein. Sera prepared against them do not, however, agglutinate cultures of streptococci. They are soluble in sodium taurocholate solution but they are not so easily dissolved as untreated pneumococci. Amoss (1925) described similar modifications of the pneumococcus, as also did Griffith (1923), and they are probably the same kind of changes as Eyre and Washbourn (1897) observed in old cultures. The organisms isolated by Wadsworth and Sickles (1927) from immunized horses suffering from endocarditis fall into the same category. It would seem, therefore, that the conclusions of Morgenroth and his co-workers may not at present be accepted. The fully equipped pneumococcus is readily distinguished from the closely related streptococcus. Even in its most degraded form it is still capable of differentiation by the bile test—especially if it be borne in mind that this may vary quantitatively (Malone, 1924), and that certain conditions such as very high acidity and high alkalinity are unfavourable to the reaction.

To sum up, while the organisms of the streptococcus group show a certain amount of quantitative variation in their activities, yet the evidence that this tends to go beyond the bounds set by the broad system of classification at present in vogue is unsubstantial.

Superinfection.

Morgenroth and his co-workers in a series of papers (Morgenroth, Biberstein and Schnitzer, 1920; Morgenroth and Abraham, 1921; Schnitzer and v. Kühlewein, 1921; Lange, 1921) attempted to show that in streptococcal infections the phenomenon of superinfection could be demonstrated. They first injected doses of 'green-producing' non-virulent variants into mice, and then at intervals from a few hours to three days tested the resistance of the animals to lethal doses of the corresponding hæmolytic streptococci. They concluded that resistance—as evidenced by time of survival after the test dose—was increased in a

few hours and continued to increase up to three days. They regard this as due to a depression of the second infection in virtue of the first. It seems rather a straining of language to call the result of the first injection an infection. Nor do the authors demonstrate that living organisms are necessary. The protocols do not suggest that the increase during the first two days is at all large nor is it regularly produced. Kuczynski and Wolff (1920) were unable to demonstrate it at all in animals tested 24 hours after the preliminary injection. There does not appear to be sufficient reason to accept these conclusions without additional evidence. Similar slight degrees of increased resistance have been demonstrated soon after injection of dead cultures in other experimental infections.

Relation of Streptococci to Rheumatic Fever.

Although Triboulet and Coyon (1897) had reported the isolation of cocci from the blood of persons suffering from rheumatic fever, and Weştphal, Wassermann and Malakoff (1899) had isolated streptococci from a case of chorea after death, yet it was in the main the work of Poynton and Paine, beginning in 1900, that led to the serious consideration of these organisms as being ætiologically related to this disease. Their view was that a diplococcus, which might show chain formation, could sometimes be isolated from the blood-stream or the heart-valves or the subcutaneous nodules both during life and after death, and that it could sometimes be demonstrated, though not necessarily in large numbers, in the various lesions. This coccus, which they termed *Micrococcus rheumaticus*, they regarded as specific, not in the sense that it was characterized by any special and peculiar biological activities *in vitro* or by any serologically distinctive features, but in virtue of the fact that when injected into experimental animals it reproduced lesions in the joints and in the heart with great readiness, but did not cause an acute general septicæmia such as was produced by the pyogenic streptococci. They were of opinion that it was not always to be found in the blood-stream or in the joint fluid, but that it gave rise to foci of infection which might be shortlived owing to the rapid destruction of the infecting organism. In these foci the bacteria would, therefore, be difficult to demonstrate. On the other hand, as for instance in lesions of the valves, the lesion might progress and the organisms multiply greatly, simple rheumatic endocarditis passing into the malignant form. In still other cases the activity of the lesion might diminish, healing by fibrosis occur and certain organisms lie dormant, to awaken into activity at a later date. This view received some support from the work of Walker and his associates (Walker, 1903 ; Walker and Ryffel, 1903 ; Beaton and Walker, 1903), and especially from Beattie and his collaborators (Beattie, 1904[1 & 2], 1907, 1910 ; Beattie and Yates, 1911, 1913). Beattie in particular stressed the fact that the blood-stream and the joint fluids rarely contained the coccus, which could only be found with any regularity in the seat of the infection, especially the synovial membranes, and further that this distribution was found to

characterize the experimental disease in rabbits. He considered the organism to be quite distinct from the pyogenic streptococci in virtue of its different effects when injected into rabbits. Beattie's conclusions as to the relation of the streptococci to the human disease were mainly based upon cultures made from bodies after death. These authors describe the organisms which they isolated, but as they consider the ordinary biological tests (fermentation) unreliable they do not stress them. But it would appear that the organisms were not all the same. Beattie (1910) remarks on the longevity of his organisms in artificial culture (3 years) and (1907) that it grows well in bile-salts—characters indicative of the enterococcus or *S. fæcalis*. Poynton and Paine, however, considered the organism difficult to grow, and Walker (1907) mentions the production of green discoloration of blood-agar, while Walker and Ryffel (1903) say that one of their strains was, in test-tube experiments upon suspensions of cells, the most actively hæmolytic organism in their experience. This evidence supports the view expressed by Andrewes and Horder (1906) that the strains isolated were not all the same and might be salivary, fæcal or hæmolytic streptococci. Beattie and Yates (1911) considered the relation of their organisms to *S. pyogenes*, but it is not clear from their work that hæmolytic activity was actually taken into account.

Some confirmation of these views is to be found in the work of Herry (1914) who reports the isolation of streptococci from the blood of 48 out of 60 cases during life. He states that repeated cultures were necessary in some cases. Similarly, Rosenow (1914[1]), Quigley (1918), Lintz (1918), Clawson (1925) and Clawson and Bell (1926) have published a considerable number of observations indicating that streptococci similar to those in the mouth can be isolated from the blood in rheumatic fever. Meyer (1901, 1902[2]), on the other hand, was unable to grow organisms from blood or joint fluid, but did isolate streptococci from the throats of cases with angina, and these produced arthritis when injected into rabbits. Small (1926) has published similar observations.

Many bacteriologists have, however, failed to obtain satisfactory evidence that the streptococci are causally related to rheumatic fever. Phillip (1903), Cole (1904, 1906), Horder (1907[1]), Schürer (1912) and de Vecchi (1912) have all failed to isolate such organisms from the blood and joint fluids during life. Bulloch and Thompson (1909) and Bulloch (1909) were unable to find any bacteria by cultivation or histological examination in the blood, valves, joint fluids or subcutaneous nodules. Their observations were made in part during life and in part after death. Swift and Kinsella (1917), in 85 cases found the joint fluids sterile, while the blood contained streptococci in 6. The serum from these 6 positive cases did not have any action upon the streptococci isolated and the organisms had no common biological or serological properties. During the past four years the writer has failed entirely to isolate any organisms from the blood of cases of rheumatic fever. Two subcutaneous nodules excised during life have also yielded no streptococci and sections of

material from several cases of rheumatic endocarditis and of the above-mentioned nodules have also been examined with entirely negative results.

That streptococci can produce joint and heart lesions in rabbits and in other animals (Shaw, 1904) is ground common to both sides in this controversy. That the property is peculiar to the organisms isolated from rheumatic-fever patients does not appear to be a fact according to the work of Cole (1904) and of Topley and Weir (1921). While the hæmolytic streptococci usually produce acute septicæmias, organisms of lower virulence or virulent strains in resistant animals may produce arthritis and endocarditis (Cowan, 1923). The same is also true of pneumococci (Wright, 1926). The valvular lesions in the latter cases appear to the writer to be identical with those produced by non-hæmolytic (or green-producing) streptococci whether of fæcal or salivary type. They are not at all like the rheumatic vegetations in man. In the joints, lesions produced by the more virulent organisms show a greater tendency to suppuration. The joint fluid may be sterile in all forms of infection. These localizations are explained by Rosenow (1914[1]) as due to a property of ' elective localization ' temporarily resident in the organism, and by Poynton and Paine and others as a peculiarity of *Micrococcus rheumaticus*. The evidence seems to suggest that it is in reality connected with the virulence of the organism, the resistance of the animal and, possibly the local conditions found in the joint, injury rendering it more susceptible (Swift and Kinsella, 1917). The joint lesions may go on to a chronic form in which, according to Beattie, the organisms may persist for a long time.

The real difficulty is in deciding whether the polyarthritis and endocarditis produced experimentally do in fact correspond to rheumatic fever in man. Swift (1924) has pointed out that in man the ' virus ' of rheumatic fever everywhere produces a reaction of the same type as the Aschoff nodule in the myocardium. The work of Bracht and Wächter (1909), Thalhimer and Rothschild (1914), Cecil (1916) and Kettle (1927) seems to show clearly that the lesion produced by ' green-producing ' streptococci in the rabbit is not the same. This, of course, may not mean any more than that the tissues of the rabbit do not react in the same way as do those of man.

With regard to the isolation of streptococci from the blood of human patients during life, we can do little more than note the discrepancy in the reports of recent investigations. Rosenow's technique involves much manipulation, Clawson's rather less. Kinsella (1917) could find no difference between results obtained by Rosenow's methods and those following the use of others. The writer's negative results in cultures from the blood of rheumatic-fever patients have been obtained with media which were suitable for the growth of streptococci when present in very small numbers in the blood of patients with subacute endocarditis (Wright, 1925). It is not considered that technical considerations are the explanation of these failures. Bacteriæmia is probably a rare occurrence in the course of rheumatic fever. There are two points which require

consideration. One is that there is evidence that in some publications, malignant endocarditis is confused with acute rheumatic endocarditis. Certainly some authors consider these conditions manifestations of the same disease, but this is by no means certain. Clinical diagnosis is not infrequently difficult in the early stages, and the writer's experience has been that in such cases of difficulty where a blood culture has been positive the subsequent course and autopsy findings have always been those of typical malignant endocarditis, while a failure to isolate streptococci has always occurred in cases subsequently proved to be rheumatic. Limited observation of such cases might lead to incorrect reports. The second consideration is that it seems probable that streptococci may be not infrequent invaders of the blood-stream from foci of infection on mucous surfaces. Lewis and Grant's observations (1923) upon the frequency of infection in patients with congenital anomalies of the aortic valve are suggestive in this connection. But accurate information as to such frequency, especially in inflammatory conditions of the mucous membranes, is not available. The writer's own observations upon the blood of patients with heart disease and other lesions do not suggest that it is common, but these observations have not included many cases of sore throat or similar conditions.

The earlier workers stressed the findings in the body after death as the main ground of their contentions in favour of a streptococcal ætiology for rheumatic fever. By far the greater number of strains of the '*Diplococcus rheumaticus*' appear to have been obtained from the dead body. Beattie and Yates (1913) in particular have recorded a large number of observations of this kind. They considered that these findings strongly supported their thesis. If their figures are considered in detail it will be found that streptococci were isolated from the synovial membranes in 20 per cent. of all cases. The writer (1925) has pointed out that similar organisms can be isolated from the heart-blood of a not dissimilar percentage of cases dead from any cause. These findings are in good agreement with the findings of other workers which are quoted in the same publication. Beattie and Yates, who dealt with chronic and acute cases, had this objection in view, but their argument does not in the writer's view go far towards disposing of it. Any attempt to prove the streptococcal ætiology of rheumatic fever by observations upon the dead body must demonstrate that the incidence of streptococci in the tissues and blood is significantly higher than in similar tissues from other bodies. 'Chronic rheumatism' and acute should not be confused; their ætiology may well be different.

There is great need of a sufficient study of the bacteriology of the tissues removed during life. Joint tissues are difficult to obtain, but a study of the rheumatic nodule, hitherto made only very incompletely, especially in the early stage of its development, might give conclusive information.

Herry (1914) attempted to show that injections of 'endotoxin' into a joint sensitized the part to streptococci intravenously injected

subsequently. This conception received a certain amount of support from the work of Faber (1915), who injected dead bacteria into the joint as the ' sensitizing ' dose. He considered the effect was specific. Swift and Boots (1923), repeating this work, came to the conclusion that the effect produced by preliminary injections in favouring the development of arthritis after a subsequent intravenous injection of living bacteria was not a specific one, and was probably due to the slight trauma produced by the first injection. But Andrewes, Derick and Swift (1926[2]) and Derick and Andrewes (1926) have drawn attention to a curious reaction which they observed in the skin of rabbits with certain ' green-producing ' streptococci, and which they consider to be allergic in character. Further work is necessary on this aspect of infections with streptococci.

The general lack of success in isolating organisms from acute rheumatic lesions has suggested the idea that toxins produced in some focus, e.g. in the tonsils, might account for the articular and other lesions. Birkhaug (1927[1]) has recently reported experiments tending to show that the articular lesions are due to a toxin produced by a non-hæmolytic, non-green-producing streptococcus which is found in the tonsils of rheumatic-fever patients, and has certain biological and serological features distinguishing it from other streptococci. This work is so far unconfirmed.

Hæmolytic streptococci can be isolated from the throat in many cases of rheumatic fever. These, according to Birkhaug, are not toxin producers, and Andrewes, Derick and Swift (1926[1]) have shown that they do not form a homogeneous group.

Sindoni and Vitetti (1924) record successful attempts to obtain a filter-passing virus from the blood, joint fluids and nasopharynx. Miller (1924) has, however, failed entirely to obtain any evidence of such a virus by cultivation or animal inoculation of presumably infected fluids. His animal experiments contradict those of de Vecchi (1912), which suggested that injections of patients' sera into rabbits produced myocarditis. In some unpublished experiments the writer failed to infect rabbits, guinea-pigs or a monkey with blood freshly drawn from an acute case.

The present position then with regard to acute rheumatism seems to be much as it was when reviewed by Bulloch (1909). The work done since that time has continued to give conflicting results, and it would appear to be fair to say that the cause of rheumatic fever is still not definitely known.

Relation of Streptococci to Subacute Endocarditis.

Although streptococci had been previously observed in the vegetations on the valves in some cases of endocarditis, it was Schottmüller who, in 1903, described the isolation of ' *S. mitis* seu *viridans* ' from the blood of patients with this disease during life. These observations were amplified in a further paper (Schottmüller, 1910[1]). This work has been confirmed and elaborated by subsequent workers (Horder, 1909 ; Libman and Celler,

1910 ; Major, 1912 ; Kinsella, 1917), so that now it is clearly recognized that this type of organism is in the great majority of cases the cause of that form of endocarditis which is generally characterized by a long continued course. The organisms isolated are most commonly of the salivary type. But Horder (1909) records the isolation of eight strains of *S. fæcalis*. The writer has recovered from the blood only one strain of this type in some 45 cases of the disease. All reports agree that the streptococci isolated from these cases are quite heterogeneous as to their biological reactions and also when tested serologically. By agglutination there may be a small amount of grouping but this is not marked. Kinsella (1917) found his strains fall into two groups by complement-fixation tests, using an antigen composed of chemically treated bacteria, while Stone (1923), who used suspensions of whole bacteria, failed to observe this. This is what might be expected in view of the antigenic construction which these organisms appear to possess.

The influenza bacillus (Pfeiffer) has been isolated from the blood of certain cases as reported by Horder (1906, 1907[2]), Smith (1908), Libman and Celler (1910) and Oppenheimer (1926), and a similar but hæmolytic organism was found in one case by Miller and Branch (1923).

Other organisms have from time to time been incriminated, especially hæmolytic streptococci, but few of these reports will bear critical investigation. Murray and Lougheed (1921) state that 66 per cent. of their cases were due to hæmolytic streptococci, but they also state that hæmolysis did not appear till after 48 hours' incubation. It is quite possible they were really observing α-type hæmolysis. Freund and Berger (1924) consider that the hæmolytic activity of the organisms may vary from time to time in the same patient according to the degree of resistance of the patient. Lehmann (1926[2]) has observed only one instance of anything like this, and in that case the organism was a peculiar form of *viridans* (α-hæmolytic or α-prime of Brown) which showed some variation from colony to colony but did not hæmolyse cells in suspension. The writer has recently isolated a similar strain. In all other cases repeated cultures from the same patient have given constant results. Both Lehmann (1926[2]) and Kastner (1918) have stated that hæmolytic streptococci may be isolated from the blood of some cases shortly before death. Howell (1922) found in the vegetations of one case *post mortem*, two types of streptococcus, one of which was hæmolytic, which differed also in other respects, and were to be regarded as different organisms. These findings are to be considered as evidence of agonal invasion by hæmolytic streptococci. The writer has produced evidence of the occurrence of this phenomenon in a small number of cases (Wright, 1925), and has pointed out that it is a relatively common occurrence, and that conclusions as to ætiology based upon uncontrolled observations upon the heart-blood or vegetations after death are extremely likely to be invalidated by this complication. There is, in fact, no good evidence that, with the exception of hæmophilic bacilli, any organisms except the non-hæmolytic or

α-hæmolytic (*viridans*) streptococci ever cause this disease. Rosenow (1909, 1910) considered that the organisms which he isolated were modified pneumococci, but the criteria which he employed were not sufficient to justify this view.

The streptococci can be isolated from the blood in a considerable percentage of cases. Figures reported have varied from 100 per cent. to nil. The details of these variations have been discussed by the writer in the above-mentioned paper. It seems that these discrepancies cannot all be attributed to differences in technique. Where different techniques have been compared upon the same group of cases (Kinsella, 1917; Wright, 1925), the results have been sensibly the same in all. Rosenow lays stress on the removal of the fluids of the blood and the use of diminished oxygen tensions. But the writer's experiments have not indicated that this elaborate method is likely to give any compensating advantages. If a sufficiently large quantity of blood be used (20 c.cm.), and it be well diluted in a broth containing sufficient sodium citrate solution to stop clotting, but not sufficient to interfere with growth (about 0·2 per cent.), it would appear that positive cultures may be obtained with ease and certainty. Plate cultures in agar are of advantage in certain cases in addition to those in liquid media.

The number of organisms present in the blood in different cases varies from 1 or 2 per c.cm. up to several thousand. As the case progresses the numbers tend to increase, but not regularly, and no conclusions as to prognosis can be drawn from the number of organisms in the blood. Leschke (1916) and Auerbach (1920) think the best time to examine the blood is when the temperature is high. There is no convincing evidence that this is so, and the writer's experiments indicate that the number of organisms in the circulating blood do not undergo great or sudden variations. Many statements are, however, on record to the effect that organisms may not be isolated from the blood at the first attempt whereas they are obtained later (Lehmann, 1926[2]). It appears clear from Libman's work (1913, 1925) and from other observations (Hemsted, 1913; Major, 1925; Latham and Hunt, 1911) that in some cases after a series of positive cultures the blood may no longer yield any organisms. These cases are, however, exceptional. In most instances cultures from the blood are either consistently negative or consistently positive.

Harbitz (1899) examined the valves of 26 cases microscopically and found bacteria in 16, but in 10 others was not able to detect any. Libman (1912) described a series of similar cases which during life did not have bacteria in the circulating blood, but which, nevertheless, had all the symptoms and morbid changes of the bacteriæmic cases. He clearly recognizes the existence of a group which, he thinks, at one time were probably of the ordinary type, but which had 'recovered' from the bacteriological point of view. He points out, however, that such a recovery is not synonymous with clinical recovery, and that, in fact, most of these cases die. The writer (Wright, 1925) has brought forward evidence which

suggests that in most of the cases of this type there are to be found in the vegetations evidences of bacterial colonization and that these cases are in fact streptococcal, but that the streptococci in the vegetations are attenuated, if not dead. It is quite clear that even a series of negative blood cultures does not exclude a diagnosis of this disease.

The presence of this type of streptococcus in the blood has, in the writer's experience, invariably meant the existence in the patient of subacute infective endocarditis. But Oille, Graham and Detweiler (1915) and Isaac-Krieger and Friedlaender (1924) have reported streptococci in the blood of patients suffering from mild forms of endocarditis. Not dissimilar evidence, on the whole contradictory to the general experience, is to be found in a paper by Biggs (1925). Libman (1925) says that 10 of his 800 cases have recovered, and other workers have reported individual cases of recovery. But the common experience is that a fatal termination within a short period is the rule.

In the serum of the patient antibodies may usually be demonstrated ; agglutinins (Kinsella, 1917 ; Wright, 1925) may be present in considerable amounts ; sometimes they may be active in a dilution of 1 in 1,000. Major (1912) and Stone (1923) have demonstrated the presence of complement-fixing substances. The agglutinins are active only upon the infecting streptococcus and closely related strains. The blood is usually bactericidal both for cultures of the infecting strain and for the organisms actually constituting the septicæmia, so far as this can be judged by *in vitro* experiment. These facts, together with the absence of suppuration in infected areas, indicate that the general resistance of the patient is of a high order.

Pathogenesis.

The original infection of the valves must result from an invasion of the blood-stream by streptococci. These are in most cases indistinguishable from those which are normally found in the mouth and upper respiratory tract, and presumably come from this situation. A few are probably fæcal in origin. Other sites may be the source in individual cases. That they enter the blood-stream from foci of infection is not improbable, but the crucial test of the serological identity of the strain in the blood with those derived from a focus of infection has not apparently been made.

Poynton and Paine (1913) and Shaw (1904) produced endocarditis in rabbits by intravenous injections of streptococci. Large doses were employed and the condition was produced with some irregularity. Since then these findings have been frequently confirmed. Rosenow (1915[2]) is of opinion that the localization of these organisms in the valves is by embolism of the vessels, and that it is determined by a certain quality of the organisms acquired in the individual from which they are obtained. This is a very labile property and is soon lost in culture, and cannot be properly observed unless cultures are made under reduced oxygen tension. It may be observed not only in organisms obtained from a lesion in the

human patient but also in streptococci isolated from periapical foci round the teeth. This phenomenon Rosenow described as the 'elective localization of streptococci'. He claims to have demonstrated in a great variety of conditions that streptococci (of the *viridans* type) obtained from patients with lesions in a given organ tend if inoculated into rabbits intravenously to become localized in the corresponding organ of that animal. The greater part of the evidence brought forward to support this view consists in the demonstration of minute hæmorrhages within two or three days after the injection, and not either in the demonstration of the bacteria or of the particular lesion in question. The hæmorrhages are considered to be caused by the organisms and the forerunners of the particular lesion. Haden (1923) and Bargen (1923) have produced some evidence tending to support Rosenow, but Henrici (1916), Moody (1916), Detweiler and Maitland (1918) and Topley and Weir (1921) find that the source of the organism has no influence upon its pathogenic effects. Detweiler and Robinson (1916) came to similar conclusions, and, further, that in some cases the deposition of the organisms in the valves is on the surface, while in others it appears to be embolic in nature.

Dietrich (1926) found, as many others had observed, that the production of endocarditis was much commoner if the animals had previously been treated with injections of the same organism. This he attributed to a sensitization of the tissues of the valves. Wadsworth (1919) noted the development of a very chronic form of endocarditis in horses undergoing immunization with pneumococci. Mair (1923[2]) produced a similar condition in rabbits. The writer (Wright, 1926) reported experiments upon this subject, and showed that, although it was possible to produce endocarditis in immunized animals, yet this occurred very irregularly, and under such conditions that it seemed more likely to be due to some slight injury than to any specific sensitization. The matter still remains undecided.

There is considerable evidence that in the human being streptococcal infection is particularly liable to occur in valves which have previously been injured by disease, or which show some congenital anomaly (Lewis and Grant, 1923). Similarly, Wyssokowitsch (1886) and Kinsella and Sherburne (1923) showed that injury, and particularly very recent injury, of the valves favoured the production of experimental endocarditis. Jochmann (1912) states, without giving details, that there may be a septicæmia in patients with chronically deformed valves without the production of endocarditis. It seems probable that a combination of recent injury and coincident blood infection may be necessary, and that the greater frequency of occurrence on the left side of the heart is due to a greater liability to slight injury, and not, as Boldero and Bedford (1924) and Rosenow (1909) suggest, to the oxygen or CO_2 content of the blood.

Once established the disease progresses with a greater or less degree of acuteness, occasionally to recovery but usually to a fatal end. The persistence of the septicæmia is to be attributed to overflow of organisms

from the infected focus in the valve (Lehmann, 1924; Wright, 1925). The organisms do not multiply in the blood-stream, but are readily destroyed. Failure to clear out this focus is apparently to be explained on anatomical grounds.

The petechial hæmorrhages produced in the skin are usually attributed to embolism. Morawitz (1921) showed that constriction of the arm led to the production of crops of these hæmorrhages and attributed this to a weakening of the endothelium due to the infection. This view has been amply confirmed by Lewis and Harmer (1926). It appears from Lossen's work (1926) that the platelets may also be reduced in the blood at the time of the hæmorrhages. It is possible that some of the glomerular lesions in the kidney may be due to similar changes in the capillaries, though they are usually attributed to emboli (Baehr, 1912; Gaskell, 1914).

Relation of Subacute Infective to Rheumatic Endocarditis.

Poynton and Paine regarded subacute infective endocarditis as essentially the same disease as rheumatic endocarditis. This view has been revived by Clawson and his associates (Clawson, 1924; Clawson and Bell, 1926; Clawson, Bell and Hartzell, 1926). They base their view on the observation in the valves of cell aggregates which they consider identical with the Aschoff node. But Thalhimer and Rothschild (1914) and many other observers including Clawson himself (1924) have remarked on the absence of Aschoff nodes in the myocardium in bacterial endocarditis. The writer failed to find any in some fifty hearts examined. In the valve lesions, at the base of the vegetations, collections of mononuclear cells similar to those in the submiliary nodule may be seen in many of the more chronic cases. But it would not appear possible to base conclusions as to ætiology on this fact alone, for the writer and R. T. Grant have observed similar cells in the lesions of swine endocarditis due to the bacillus of swine erysipelas. Experimental endocarditis in the rabbit is usually acute—lasting not more than 14 to 21 days, and neither in the valve lesions nor in the myocardium can cell collections like the Aschoff node be demonstrated.

Reye (1914) considered that he had been able to demonstrate streptococci histologically in every one of a group of cases of verrucose endocarditis, but Bartel (1901) thought that many of the cases of terminal verrucose endocarditis were not inflammatory and the bacteria seen in these were often accidentally and not ætiologically associated. The writer's own observations support the latter view. Even large vegetations occurring as a terminal phenomenon may be entirely free of bacteria.

Dental Caries and Periodontal Infections.

Dental Caries.

Much of the earlier work upon the subject of dental caries consists of morphological descriptions of the organisms to be found and catalogues a

considerable number of such bacteria. The conclusion generally drawn was that dental caries is not a specific bacterial infection of the teeth, but may be caused by a variety of organisms. The more recent workers are still divided in their views. The organism described by Goadby (1903) as *B. necrodentalis* has been found to be present and considered of importance in many cases. Howe and Hatch (1917) considered that *B. acidophilus*, which Hilgers (1924) has shown to be serologically identical with *B. necrodentalis*, was the most important bacterial agent in producing caries of teeth. Two distinct types of this group of organisms were described by McIntosh, James and Lazarus Barlow (1922, 1924) under the names *B. acidophilus odontolyticus* types I and II. They grow best in an acid medium (pH 6). The individual bacilli of type I are long and thin and they tend to show a degree of ' palisade formation '. Those of type II are short, form chains, and may show coccal forms. They occur in 20 to 30 per cent. of normal mouths. Individual strains differ in their fermentative capacities. Serologically there is a certain amount of interrelationship between different strains. According to Niedergesäss (1915), although cultures from the superficial part of a carious focus may yield a variety of organisms, the deeper the source of the material the more do streptococci predominate and in the deepest parts they may be present in pure culture. Baumgartner (1913) and Heim (1925) came to the same conclusion, the former from histological study of carious areas. Finally Clarke (1924) reported that he was able to isolate *B. acidophilus* only from large or shallow foci. From the deepest parts of the process were obtained streptococci which grew slowly, did not alter blood-agar, and showed bacillary forms in acid media. From this characteristic he terms the organism *Streptococcus mutans*. It fermented the carbohydrates lactose, glucose, mannitol, saccharose, raffinose and inulin but not dulcitol. The group is serologically homogeneous. It is clear that *B. acidophilus odontolyticus* type II and this streptococcus have a certain degree of morphological resemblance to one another. Maclean (1927) has demonstrated quite clearly that they may be differentiated completely by biological and serological methods. He has confirmed Clarke's findings and has been unable to find *B. acidophilus* in pure culture in the dentine although *S. mutans* was found there in pure culture in several cases. Both groups of workers claim to have produced changes in teeth *in vitro* in the presence of cultures, but no evidence of production of the condition in animals, even in those fed on deficient diets (Maclean), is forthcoming. The available evidence suggests that both *B. acidophilus* and streptococci are frequently found in carious teeth and that in the areas where the condition is apparently in its earliest stages the streptococci predominate. The ætiological relationship of these organisms to dental caries is an open question.

Pyorrhœa Alveolaris.

The multiplicity of organisms in the pus from this condition was described by Goadby (1907) and by many since. Glynn (1923) and

Thompson (1925) have stressed the importance of streptococci, and the latter has reported isolation of pure cultures of these organisms from the deeper parts of the lesion. Fisher (1927) also considers that streptococci can be demonstrated more deeply than other organisms in the tissues surrounding the lesion. On the other hand, Eyre and Payne (1910) think the streptococci are unimportant and that different organisms are the responsible agents in different cases. It cannot at present be affirmed that the condition is bacterial in origin or in particular that any given organism is the common cause. The streptococci isolated are of the type commonly found in the normal mouth.

Apical Abscesses.

Acute periapical inflammation with suppuration may apparently be caused by pyogenic organisms, but there occur round a number of apparently healthy or carious teeth areas of infiltration with or without accompanying rarefaction or sclerosis of bone, which have attracted great attention as possible sources of infection, giving rise to general disturbance of health or particular lesions in different parts of the body. Hartzell and Henrici (1915) have shown that from the great majority of such ' abscesses ' streptococci of a non-hæmolytic type which fail to ferment mannitol may be isolated. These results have been confirmed by Fraser (1923), who further observed that the streptococci so isolated were not a homogeneous group when tested serologically. Haden (1926) studied a large number of normal and of ' pulpless ' teeth and found streptococci at the apices of the latter very much more commonly than from vital teeth. Hartzell and Henrici grew no organisms from the apices of seven normal teeth. Since the organisms isolated are not distinguishable from those of the normal mouth, the significance of such cultures might be questioned. Berwick (1921) was apparently at great pains to avoid contamination, but his results agree with those recorded above. It would appear that these findings may be accepted. But the evidence that these foci are of importance in determining lesions elsewhere in the body is mainly clinical and by no means conclusive.

Relation to Other Conditions.

Attention has been directed by Rosenow (1919), Tunnicliff (1918, 1920[1]) and others, to the green-producing streptococci as important factors in the production of *influenza*. Later work by Jordan (1919) showed that the so-called ' Mathers coccus ' associated with influenza could not be distinguished from the ordinary mouth streptococci. The suggestion of Rosenow and his colleagues (1918) that similar organisms are the cause of *poliomyelitis* is contradicted by the evidence produced by Bull (1917). Similarly, there is no satisfactory confirmation so far forthcoming for the view that streptococci are the cause of *gastric ulcer*, *appendicitis*, *cholecystitis* and a variety of diseases of the central nervous system.

Tunnicliff (1917) has reported the isolation of streptococci from the blood of *measles* patients in the pre-eruptive and eruptive stages of the disease. At first the coccus grows anaerobically but later in the presence of oxygen. It is also found in the throat. At certain stages it may pass through a filter-candle. The organism is specific for the disease and is pathogenic for the rabbit, dog, mouse and rat but not for the guinea-pig (Tunnicliff and Moody, 1922). A serum prepared against the cocci and filtrates from cultures is said to be an efficient prophylactic against measles (Tunnicliff and Hoyne, 1926). This work stands in opposition to much previous experiment, indicating that the cause of measles is a filterable virus and lacks independent confirmation.

Treatment.

Subacute Infective Endocarditis.

Various writers have reported attempts to treat this condition by means of vaccines, sera and whole blood transfusions. Billings (1909) used both vaccines and transfusions of normal blood without effect. Latham and Hunt (1911) record a recovery with disappearance of the bacteriæmia after administration of a vaccine by the mouth. Horder (1904) used an autogenous vaccine and a serum prepared against the organism isolated from the patient's blood in one case without success, but Hemsted (1913) records in detail the recovery of one case treated in this way. Wordley (1924) treated one non-bacteriæmic case with immuno-transfusion and the patient apparently recovered. The course of antibody production following immuno-transfusion was studied by Howell, Porter and Beverley (1926) in one case which was treated in this way without success. The writer has had the opportunity to see three cases treated with serum prepared against the organism isolated from the patient's blood without any beneficial effects. In one other case transfusion with the blood of a donor immunized with the infecting strain was similarly ineffective. Major (1925) has recorded the recovery of one case following intravenous injections of gentian violet. The writer has tried this method in one case without benefit. On the whole, measures of treatment so far adopted have been singularly unsuccessful.

Rheumatism.

The question of the vaccine therapy of rheumatism, both acute and chronic, and of other conditions considered above, is at the present time difficult to discuss. As pointed out, the ætiology is quite uncertain, so that it is not clear whether the results obtained are to be regarded as specific or non-specific. Further, the results are presented in such a way that it is quite impossible to form any valid opinion upon the efficacy of the methods adopted.

The Bowel Streptococci.

By J. Henry Dible.

The recognition of cocci of the genus streptococcus in the stools was doubtless made as soon as the practice grew up of examining these bacteriologically by the Gram method of staining ; so that their discovery belongs to the dark ages of bacteriology and was probably made independently by a number of observers at about the same period. A search for the first claimant to their recognition would, therefore, be a difficult and rather futile affair. The attracting of attention to such fæcal organisms as possible pathogenic agents is generally credited to Hirsch (1897) and Libman (1897) who drew attention to the presence of streptococci in the stools in cases of summer diarrhœa, and noted the occurrence of both long- and short-chained forms. They gave the name of *S. enteritis* to this organism and the title was also adopted by Escherich (1899) for a chain-forming organism found in cases of infantile enteritis. It seems from a perusal of the literature that these authors were dealing with definitely pathogenic cocci in some instances. It must be recognized that the descriptions of these organisms were given before the introduction of the more modern methods of investigating the streptococcus group, and that they are therefore inadequate, and in consequence exactly what the described organisms were must remain largely a matter of speculation.

At about the same period, Thiercelin (1899), in France, described a short-chained, or diplococcal, bowel streptococcus under the name of *Enterococcus*. He recognized it as a normal inhabitant of the intestinal canal, and differentiated it from the bowel streptococci mainly upon morphological grounds. The same, or an indistinguishable organism, had been previously noted by Escherich (1886) in the stools of young infants and designated *Micrococcus ovalis*. Thiercelin considered that his organism was capable of becoming pathogenic, and a causal agent of certain diarrhœas, biliary infections and cases of appendicitis. Tissier (1900) paid a good deal of attention to the enterococcus in the study of the fæces of infants, and differentiated it from the *Streptococcus enteritis* upon morphological grounds, its greater hardiness in culture, and its more rapid action in clotting milk. He considered the enterococcus synonymous with the *M. ovalis* of Escherich. In his hands neither of these two streptococci showed much pathogenicity. The organism became generally recognized in French bacteriological literature, and the name *Enterococcus* was adopted and has persisted constantly ever since. The impression conveyed by various descriptions, too numerous to detail here, is that the organism was generally considered unique—a single species—and amongst other properties its general vigour in cultures and marked viability are most constantly commented upon.

The German literature in the main followed Escherich (1886) and Hirsch (1897) and Libman (1897). Schmitz (1912) suggested that the

frequency of the presence of the enterococcus was much exaggerated by French workers, stating that he had never encountered the organism in normal stools. In view of the results to be recorded later in this article, it is difficult to understand how such a conclusion was reached.

The recognition of a fæcal group of streptococci on grounds other than those of morphology and location came as a result of the introduction of the sugar fermentation tests as criteria for differentiation by Gordon (1904). This was followed by an extended examination of the main features of a large number of organisms obtained from the fæces by A. C. Houston (1905). Matters took a yet more definite turn with the publication of the pioneer paper of Andrewes and Horder (1906), who, combining ' Gordon's tests ' with a consideration of the action of the organisms upon blood, divided the whole family of streptococci into seven large groups, one of which they considered characteristic of the stools and designated *S. fæcalis*. An important feature of this work was the stressing of the fact that classification of these organisms was an affair of groups, each arranged about a central type and constituted by slightly differing organisms, which were considered to be variants either by excess or defect. At the same time the necessity remained of insisting on one essential feature for each group, and, since intermediate forms closely approached one another, the classification, though admittedly a considerable step forward, was vulnerable in this respect. The characters of the central type of the *S. fæcalis* group according to Andrewes and Horder are these :

' It is a short-chained organism and renders broth uniformly turbid. It grows readily at 20° C. and forms sulphuretted hydrogen in broth cultures. It has no hæmolytic power and little virulence, but produces a positive reaction to all Gordon's tests except raffinose and inulin. That is, it clots milk, reduces neutral red, and ferments saccharose, lactose, salicin, coniferin and mannite. The mannite reaction is specially characteristic of this intestinal type ' (Winslow, 1908). It is to be noted that the classification of Andrewes and Horder did not admit the existence of a *S. fæcalis* which failed to ferment mannitol, this being the critical test which they established for this group. The description of Andrewes and Horder's *S. fæcalis* as given above applies exactly to the commonest type of streptococcus found by A. C. Houston (1905) in 300 fæcal strains, 13 per cent. of which had these characters. Houston also found that 32 per cent. of his cocci fermented raffinose, whilst only 4·6 per cent. fermented inulin.

From a more extended survey of the literature, of which only the more historically important papers have been noted here, it will be seen that general recognition was granted on all sides to the existence of a special fæcal streptococcus, or group of streptococci, but since the question had been approached from different angles there was no complete agreement as to the characters of these, and detailed examination of the literature assembled about the names *Micrococcus ovalis*, *Streptococcus enteritis*,

Enterococcus and *Streptococcus fæcalis* shows that even where common ground had been explored the results recorded by different observers were often mutually contradictory, and upon hardly any points was there unanimity of opinion. During the War much attention was directed towards this group of organisms, both as a possible source of infection of suppurating wounds and also as causal agents of many of the ill-defined pyrexial conditions which abounded. The chief contribution to the latter aspect of the subject was that of T. Houston and McCloy (1916) who stated that they had isolated the organism from the blood and urine of patients suffering from trench fever and clinically allied conditions. Whilst their claim to its ætiological significance in these conditions has not been admitted, their paper is of importance, since it contained a comprehensive description of the organism and drew attention to its remarkable power of resisting destruction by exposure to heat.

In view of the confused state of the literature, Dible (1921) undertook an extended study of the streptococci to be found in the fæces, utilizing both normal and pathological stools, and applying most of the tests and criteria for differentiation which had been put forward by various workers up to that time. A large number of strains was examined which were isolated by many different procedures, to exclude as far as possible the influence of selection. The characters of these strains were then analysed statistically to ascertain if, and to what degree, the various properties studied were associated in individual organisms. The general conclusions which emerged were that the fæces contained both long-chained varieties, of the type of *S. enteritis*, which were certainly, in large measure if not entirely, surviving salivary organisms having features closely akin to those of the mouth and throat streptococci. The characteristic organism of the fæces was found to be a diplococcal or short-chained organism, agreeing in these and certain other particulars with the enterococcus of French workers, and having rather diversified biological characters but with a marked tendency for the appearance of certain of these and the absence of others which enabled a fairly well-defined group to be established. These conclusions were summarized as follows :

1. The *Streptococcus enteritis* comprises the large group of chain-forming streptococci of the fæces, many of which are undoubtedly surviving salivary types.

2. The *Streptococcus fæcalis* of Andrewes and Horder comprises the group of the enterococcus and possibly some of the above-mentioned types. It omits many true enterococci, since non-mannitol-fermenting variants are not recognized.

3. The characters of the *Enterococci* have been described, as well as those of certain other fæcal streptococci. It would appear that the former comprise a well-defined group, worthy of specific rank within the streptococcus genus.

Later work by a number of investigators has generally confirmed these findings (Kendall and Haner, 1924 ; Ayers and Johnson, 1924 ; Bagger, 1926 ; Dudgeon, 1926), whilst somewhat extending our knowledge of the enterococcus group. It has been stated by some workers (Dudgeon, 1926; Wordley, 1921 ; Meyer, 1926) that hæmolytic streptococci are common denizens of the fæces and that certain otherwise typical enterococci are hæmolytic. Meyer and Schönfeld (1926) applied the æsculin test of Harrison and van der Lack (1909) to the recognition of the enterococcus and its differentiation from *S. viridans,* and reached the conclusion that a positive reaction to this test was an obligatory characteristic of the organism, as was also resistance to bile (Weissenbach, 1918). In their hands the characters of thermo-resistance, the capacity to split mannitol, a diffuse growth in broth, and an oval shape of the cocci, were also found to be present in a high proportion of strains.

The Common Characters of the Fæcal Streptococci.

In examining in detail a large number of cultures of streptococci from the stools, 257 strains in all, the writer found that the organisms fall broadly into two classes—true streptococci and enterococci. The majority of the former have no special characters differentiating them from streptococci from other sources, but the latter constitute a group which are characteristic of the fæces by the constancy of their presence, and are to be distinguished from other streptococci by their special characters and will be referred to as *enterococci.* It may be said at once that in the present state of our knowledge it is impossible to lay down definite criteria for the recognition of these organisms, in the way in which such criteria can be laid down for the typhoid bacillus or the tubercle bacillus. Amongst the non-hæmolytic bowel streptococci innumerable varieties can be recognized, and the biological tests upon which we at present rely for enlightenment do not serve us well. From time to time rigid criteria have been proposed for the fæcal group. Andrewes and Horder (1906) insisted, as a matter of practical necessity, that their *S. fæcalis* should ferment mannitol ; Weissenbach (1918) considered that the enterococcus could be differentiated from the pathogenic streptococci by its ability to grow in bile-glucose-peptone broth, which inhibited the latter ; the writer (1921) utilized the heat-resisting powers of the organism as an essential criterion ; and Meyer and Schönfeld (1926) require that it shall be able to split æsculin. Now, the difficulty is that to each of these tests exceptions occur, so that it becomes necessary to recognize the existence of variants showing departures in some respects from the type organism. In general, however, the characters mentioned above are present positively, and, which is of great practical moment, they are characters rare in other varieties of streptococci and in these are scarcely ever met with in combination one

with the other. The frequence of these characters is brought out in the following table (Table V):

TABLE V.

Showing in percentages the distribution of characters in populations of undoubted enterococci possessing the attribute denoted in the column on the left (Author's observations).

	Mannitol Fermentation	Diplococcal Form	Heat Resistance	Æsculin Splitting*
	Percentage of Strains Positive.			
Mannitol - fermenting strains (108)	=	96	92	91
Diplococcal strains (177)	58	=	81	86
Heat - resistant strains (151)	66	95	=	90
Æsculin-splitting strains (74)	41	89	80	=

* The æsculin test was not carried out on so large a series of organisms as the examination of the other properties : hence the percentages given under this heading only represent the incidence in about a half of the total strains examined.

It is of interest to know to what extent these properties are linked and to what degree one conditions another. When the matter is mathematically analysed and association coefficients worked out, it is found, in the strains which have been studied, that heat resistance and failure to form chains in broth are the properties most closely associated, followed by heat resistance and mannitol fermentation and heat resistance and the faculty of splitting æsculin ; there is also a high degree of association between diplococcal form and mannitol fermentation in these fæcal strains. It follows from what has just been said that whilst a large proportion of the organisms found in the stools can be definitely identified as enterococci, a small proportion are of more doubtful status.

THE SPECIAL CHARACTERS OF THE ENTEROCOCCI.

These organisms form a group of short-chained streptococci characteristic of the fæces.

Morphology.

The organism grows well upon agar, producing colonies which are definitely larger than those of the *S. pyogenes*. The average diameter of discrete colonies after 24 hours' incubation is about 0·75 mm. ; this being approximately twice the size of the colonies of pyogenic streptococci grown under the same conditions. Where the colonies are well spaced they are often 1·0 mm. in diameter. The colonies are round, sharply contoured

and convex-lenticular; viewed under a low magnification they are uniformly and finely granular. Growing in fluid media the organism produces a uniform fine turbidity with little or no deposit in the bottom of the tube. It grows very heavily in glucose broth but will flourish in most media and over a wide range of pH variation. In pure serum growth is minimal and in serum broth there often appears to be a clumping or agglutination of the organisms. Where heavy inoculation is made from a broth culture the resulting growth upon agar appears as a uniform film, resembling a growth of *B. typhosus:* a feature which follows from the uniform distribution of the cocci in the broth culture. The enterococcus grows well in gelatin, either at 22° C. or at room temperature. On plates the colonies resemble those formed upon agar. The gelatin is not as a rule liquefied, but certain exceptional strains cause liquefaction.

The statement frequently occurs in the literature that this organism grows best under anaerobic conditions, or that a period of cultivation under anaerobic conditions is necessary before growths can be obtained aerobically. This phenomenon has not been encountered by the writer. Possibly the statement has its origin in a recommendation by Thiercelin (1902) that a preliminary cultivation of fæcal material under anaerobic conditions favoured the isolation of the enterococcus; the author tested this statement (1921) and did not find the method specially selective. Bagger (1926) found that all his strains were facultatively anaerobic, but that the growths under these conditions were no more luxurious than under aerobic circumstances. He did not encounter any strains requiring preliminary anaerobiosis.

Microscopical appearances. The enterococcus is characteristically a lanceolate diplococcus, the elongation of the units, however, being less marked than in the classical pneumococcus. In certain media, and with certain strains, a halo appears around the organisms, but no capsule has been demonstrated. There is little doubt that this organism has been frequently mistaken for the pneumococcus where morphological appearances have alone been relied upon. In broth cultures, short chains are often seen, but even so the diplococcal form tends to predominate, and the chains rarely exceed six or eight elements. The form of the organism is little influenced by the composition or reaction of the medium. In films made from agar cultures the cocci appear in staphylococcal formation with no very definite arrangement of individual units, and often no suggestion whatever of chain formation. Where they are more sparsely scattered a number of diplococci may be seen, and not infrequently the individual cocci are definitely lanceolate. This character is not invariable but when seen is very typical.

A good deal of stress has been laid, especially amongst the older French authors (Macé, 1912), upon the pleomorphism of the organism. Whilst a certain amount of involution may be observed in some strains and in old cultures it cannot be said that this is by any means a characteristic feature of young cultures upon the more ordinary media.

Biological Properties.

The vitality of the organism is very great ; a feature which is not shared in by the ordinary pathogenic streptococci. Cultures in gelatin and serum-broth have been found to be alive after the lapse of two years. The drying up of the medium does not necessarily result in the death of the organisms and in such cases they can often be recovered from the inspissated material.

Fermentative reactions. The enterococci are amongst the most actively saccharolytic members of the streptococcus group ; many strains split glucose, saccharose, lactose, salicin, mannitol and sorbitol. Dulcitol is not decomposed. Raffinose is attacked sometimes and inulin occasionally. Litmus milk is as a rule acidified and clotted, with loss of colour in the deeper portions of the tube, the result being a firm white clot with a pink portion at the top, looking like an ice cream. This appearance is rather characteristic but is not a constant one. The only sugars which are of much significance as differential agents are mannitol and raffinose. The fermentation of mannitol is a positive character which is of importance when present as it is in a little over 70 per cent. of strains ; the fermentation of raffinose, which is only shown by about 13 per cent. of strains, is a negative characteristic of some value since this sugar is split by a high percentage of salivary streptococci and by many of the chain-forming organisms of the fæces. It may be added that the sugar reactions show little tendency to change as a result of preservation of the organism in the ordinary laboratory media.

Heat resistance. This property was first brought into prominence by Houston and McCloy (1916), though other observers had previously noted thermo-resistant cocci in the stools (Logan, 1914) and in milk (Ayers, Johnson and Davis, 1918). The test as applied by the author (Dible, 1921) has proved a reliable guide to the identification of the organism in human material, since the difference between the thermo-resistant and thermo-sensitive forms of streptococci is a sharp one. The latter are killed in about 5 minutes at 60° C., whilst the former will survive such a temperature for 30 minutes, and, as shown by Bagger (1926), the majority of strains will survive an hour's exposure, provided that the pH of the broth is maintained at about 7·5. In practice the test may be very simply carried out by exposing a small quantity of a neutral broth culture of the organism, contained in a Pasteur pipette, to a temperature of 60° C. for 20 minutes. Like other tests for the identification of streptococci, it is probable that this is not an absolute one. In the writer's experience, however, it is more constantly positive in organisms having all the other recognized characters of the enterococcus than any other single reaction, and in consequence he considers it by far the best single test available.

Hæmolysis. In the experience of the writer the enterococcus never produces hæmolysis of blood-cells. The matter, however, is stated differently by a few authors. Dudgeon (1926) describes hæmolytic enterococci, but the technique employed is a peculiar one (Dudgeon,

Wordley and Bawtree, 1921), and possibly gives rise to changes which would not occur where the test is carried out in the manner advocated by McLeod (1912), which only indicates the presence of a readily diffusible hæmolysin.

K. Meyer (1926) reports that he has found a few strains of enterococci (9 in 300 studied), which took on hæmolytic characters after repeated cultures on blood-agar plates. Their colonies then produced a zone of hæmolysis and a filterable hæmolysin in exactly the same way as *S. pyogenes* does, although the other characters of the organisms remained unaltered. Five of these strains were obtained from pathological conditions. He also obtained a hæmolytic strain by passage through a rabbit. P. Lesbre (1926) claims to have rendered a strain of enterococcus hæmolytic by growing it in pus : there are insufficient details of the hæmolysis technique employed. It may therefore be said in summary that there is some evidence that enterococci may occasionally produce hæmolysis, but it must be emphasized that if this does occur it is an extremely rare phenomenon, and K. Meyer (1926), himself, states that the property is not found in freshly isolated strains.

Green coloration. When the organism is grown on blood-plates no green coloration is produced ; but on heated blood-agar there is in between 25 and 50 per cent. a slight green coloration most often limited to the area covered by the colony.

Growth in bile. The organism is insoluble in bile or in the bile-salts. On the other hand, it grows readily in media, such as McConkey's, which contain these substances. Weissenbach (1918) has advocated a peptone-broth medium containing 10 per cent. of bile as a test medium for this organism. Meyer and Schönfeld (1926) have generally confirmed this, but found that a certain number of *viridans* strains would grow in the 10 per cent. bile-peptone water, and that a medium containing twice this quantity of bile was preferable, though even this quantity did not inhibit all the strains of *S. viridans*. The author has no personal experience of these media.

Æsculin splitting. The action of bacteria in bringing about the hydrolytic splitting of this glucoside into glucose and æsculetin, which may be made evident by the black colour developed by the latter in the presence of iron salts incorporated into the medium, was first noticed by Beijerinck and used in bacterial differentiation by Harrison and van der Lack (1909). Rochaix (1924) found the test of general use in separating enterococci from streptococci proper, and Meyer and Schönfeld (1926), applying it to a considerable number of strains of enterococci and streptococci, concluded that it was the best available criterion for their differentiation. They found that only 6 out of 82 *viridans* strains gave the reaction, whilst only 1 out of 18 hæmolytic strains did so ; 91 out of 92 of their enterococcus strains reacted positively. C. Weatherall and the author have examined the utility of this test for the purposes of this article (vide Table V.) and found it positive in 59 out of 61 strains which were heat-resistant,

and on other grounds conformed to the conception of enterococci as here stated. They also found it positive in 15 other strains from the stools which were heat-sensitive or chain formers. The test was negative in 25 pyogenic strains, of which the majority were hæmolytic. There is therefore a fairly close association between this property and the others commonly found in the enterococci and it may be fairly concluded that the test is rarely negative amongst them. It would seem advisable that in employing it use should be made of the fluid medium containing bile-salts. These play no essential part in the reaction, but serve to inhibit the growth of streptococci other than enterococci. If these substances are omitted many non-enterococcal organisms give the test.

Other biological characters. Many of the organisms produce a small quantity of sulphuretted hydrogen in lead acetate broth. They do not produce indole in peptone water and have no power of decomposing urea.

Summary. The biological characters of the commoner types of enterococci are summarized in the following table:

TABLE VI.

	Clotting of Milk	Mannitol	Raffinose	Æsculin	Heat Resistance	Action on Blood-Cells
Type organism	+	+	−	+	+	−
Less common variants	+	−	−	+	+	−
	−	+	−	+	+	−

Other sugars have been omitted since variations in action upon them do not seem to be of great significance. For details see Dible (1921) and Bagger (1926).

Pathogenicity for Laboratory Animals.

Most modern workers are agreed that this is low. The author (1921) tested 69 strains from the stools on mice, by the subcutaneous injection of 1·0 c.cm. (less frequently 0·5 c.cm.) of a 24-hours' serum-broth culture of the freshly isolated organisms; 56 of these were found to be entirely devoid of pathogenic action either immediate or delayed; 13 organisms showed some degree of pathogenicity, of which only 7 were enterococci, the others being true streptococci. 5 enterococci were examined for pathogenicity towards rabbits; in all cases the results were entirely negative, even when large doses, e.g. 5·0 c.cm. of a 24-hours' serum-broth culture were injected intravenously. Attempts at exaltation of virulence by passage failed. Bagger (1926) found that 1·0 c.cm. of a broth culture, injected intraperitoneally, killed 16 out of 24 mice. With rabbits intravenous injection of 1·0 c.cm. of a broth culture was sometimes fatal; a subcutaneous injection might cause abscesses. Passage experiments

failed and no virulence was found for guinea-pigs. It is noteworthy that about two-thirds of Bagger's strains were obtained from cases of acute appendicitis and that 4 out of the 7 pathogenic strains obtained by the author were isolated from the stools of a case of typhoid fever. It is possible that the virulence of the organisms, or the preponderance of pathogenic forms, may be increased in pathological conditions in the intestines.

Occurrence.

The organism is constantly present in the stools: it is the most frequent member of the streptococcus family in this situation and will always be found when a large number of organisms from any single stool is examined.

Selective Method of Isolation.

Advantage can be taken of the ability of the organism to resist heating. A tube of broth should be inoculated with fæces, and, after incubating for 18 to 24 hours, heated for half an hour to 60° C. Some fresh broth is then added and the tube reincubated and afterwards plated upon agar. An abundant growth of enterococci results: most other non-sporing organisms are killed.

Agglutination.

Bagger (1926) reports that the results of attempts at agglutination were irregular, inconsistent and disappointing. They yield no hope of a serological classification of enterococci. Absorption experiments proved equally fruitless.

Other Streptococci of the Fæces.

In addition to the enterococci just described cultural examination of the fæces will reveal a group of chain-forming streptococci. A large proportion of these fail to effect hæmolysis or to produce colour change upon blood-agar, and would be grouped in the *salivarius* or *mitis* types under the older classifications. They are thermo-sensitive, have a much greater tendency to split raffinose and to a lesser extent inulin than have the enterococci, and they very generally fail to ferment mannitol. As a rule they do not affect æsculin. It is probable that many of these organisms are mouth and throat strains which have survived the passage of the gut.

Little is known of the distribution of these different types throughout the intestine or the factors which influence this. The enterococci are rare in the mouth, and Gordon (1904) failed to encounter any mannitol fermenters in examining 300 strains from the saliva. The writer (1921) examined the saliva of five healthy individuals by the selective method of heating and enrichment and failed to find any enterococci in three of these. The effects of diarrhœa and constipation seem to be to alter the streptococcal flora both quantitatively and qualitatively. Solid stools give a slightly higher ratio of streptococci to coliform organisms than do loose stools, and in diarrhœa the proportion of mouth and throat types appears in general to be higher than where the stools are solid.

Where colonies are selected at random from ordinary agar plates the finding of hæmolytic streptococci is a very unusual occurrence. Davis (1920) followed out the fate of hæmolytic streptococci given by the mouth to rabbits, which normally do not show these in the fæces. He found that the administered organisms were present in the fæces the next day but rapidly disappeared. Moody and Irons (1920) made kindred observations on patients suffering from scarlet fever, in whom hæmolytic streptococci were present in the throat. They succeeded in obtaining 22 hæmolytic strains from 309 stools. It is therefore evident that such organisms may occur in the fæces and under certain conditions be present in moderate numbers. Where blood-agar plates are used the search for these organisms is much simplified. Dudgeon (1926) and his associated workers (Wordley, 1921), using the special technique of the former, have obtained hæmolytic cocci with considerable frequency. Using Dudgeon's technique for plating, and employing blood-agar, C. Weatherall and the writer have occasionally isolated hæmolytic streptococci from normal stools. These are chain-forming organisms, which in general fail to ferment mannitol, give no reaction with æsculin and are heat-sensitive. They are not to be distinguished by their biological properties from the hæmolytic pyogenic streptococci.

Pathogenicity of the Enterococcus for Man.

The earlier observers attributed a pathogenic role to the bowel streptococci in enteritis and infantile diarrhœa. Their observations were founded upon the presence or predominance of such organisms and have not been confirmed by more exact observations, although there would seem to be little doubt that some of the chain-forming types described by Hirsch and Libman were definitely pathogenic. The enterococcus has been described again and again in appendicitis and septic abdominal conditions, but there seems no direct evidence to implicate it in any causal role, and it would be hard to conceive how so ubiquitous a denizen of the lower intestine could be absent from such lesions.

Macaigne (1920) devotes a chapter to this organism and the infections which it causes in the *Nouveau Traité de Médecine*. He contends that the organism has a causal role in many cases of inflammation of mucous surfaces, and puts forward, as evidence of this, the greater pathogenicity for laboratory animals of strains isolated from these conditions. He also states that the organism may be causal in catarrhal jaundice, angiocholitis, liver abscess and Hanot's cirrhosis. It is obvious that many of these statements are no more than expressions of pious opinion and scientific evidence for them is lacking. In justice to the author it should be said that he remarks that insufficient observations have been collected to determine the importance or frequency of its action, or what are the special features of this. There are also present in the literature a host of papers of a minor type noting the presence of this organism in various pathological conditions. Most of these are based upon the simple finding of organisms

having the morphological characters of the enterococcus in sites in which a mixed flora might be present, and will not stand critical examination. The writer observed a case of septicæmia in which an organism of this type was isolated both from the blood and urine : this, however, was before the special characters of the enterococci were worked out, and the exact proof of its nature is lacking. The case terminated favourably. It may also be added that two reputed strains of rheumatic cocci which were submitted to the writer were found to be heat-resisting enterococci, and one strain of streptococcus out of six isolated by H. D. Wright from the blood in ulcerative endocarditis was of the same nature. The organism was frequently isolated from wounds in the War by a large number of observers, the majority of whom commented upon the coexistence of short-chained or diplococcal organisms of this type and long-chained hæmolytic streptococci. It appears probable that in most cases the enterococcus played only a minor role in wound suppuration.

The enterococcus is very frequently met with in the bladder in cases of cystitis, in which it is a commonly encountered type of streptococcus, and is usually found in association with one of the varieties of *B. coli*. It is commonly present in the normal urinary meatus, and in many cases of pyuria it may be a secondary invader.

In cases of cholecystitis and gall-stones these organisms have been frequently described. Being possessed of the power of growth in pure bile their persistence might be expected, whilst the chances of such organisms reaching the bile-passages must be considerable. It is, however, an unanswered question to what extent they are truly causal of these conditions.

On the whole the evidence that these organisms play at all a frequent role in disease processes is somewhat scanty and unsatisfactory. Where they have been encountered in pure culture, and under conditions in which the probability of their causal role is high, their true nature has been by no means always proved. With better knowledge of this group it may be expected that future work will speedily reveal what their exact importance in human pathology may be. It is probable that the organisms become established with considerable frequency in existing pathological conditions.

Relationship of the Enterococcus with the Lactic Acid Streptococci.

The organism is an active producer of acid in milk and the majority of strains provoke acid clotting. The *Bacterium lactis acidi* of dairy bacteriologists is described as a Gram-positive oval coccus, sometimes forming chains, but usually appearing in the milk as a lanceolate diplococcus. It produces a uniform turbidity in broth, clots litmus milk with the same appearance as the enterococcus produces (p. 130), and ferments cane sugar, lactose and mannitol. These properties, it will be seen, are shared with the enterococcus : it is also noteworthy that many

investigators of milk bacteria have found varieties of streptococci showing a marked resistance to the pasteurization methods employed in preserving milk. Although many varieties of streptococcus can be distinguished in milk (Orla-Jensen, 1919) the enterococcus-like strains form an important proportion of these. It is from fæcal contamination that the cocci are most frequently introduced into milk, and it has been shown (Orcutt, 1926) that the enterococcus is a normal denizen of the alimentary canal of bovines. The relationship of the fæcal streptococci to the lactic acid bacteria has been specifically investigated by Ayers and Johnson (1924), who found their fæcal organisms (mostly enterococci) to be indistinguishable in their major characters from typical lactic acid cocci from milk, and concluded that the two forms were ' either closely related or identical '. C. Weatherall and the writer have investigated 22 strains of milk streptococci and found 12 of these to be heat-resistant non-hæmolytic diplococci, whilst 15 were positive to the æsculin test. None of these strains fermented mannitol, which from the observations of others also would seem to be a less frequent property in milk and bovine intestinal strains than in those of human origin. It would appear that the conclusion that the common *Streptococcus lactis* (*S. lacticus, Bacterium lactis acidi*, &c.) is either ' closely related or identical ' with the enterococcus is therefore justified : it may well be that Pasteur's original ' ferment lactique ' was such an organism.

The Anaerobic Streptococci.

By A. L. Taylor.

Historical.

Our knowledge of the anaerobic streptococci dates from the work of Veillon (1893), who described under the name of *Micrococcus fœtidus* a strictly anaerobic coccus which he isolated from cases of Ludwig's angina, perinephric abscess and suppurative bartholinitis. This coccus grew in the form of diplococci and in short chains and produced gas and fœtid odour in culture. Later similar organisms were isolated by Rist (1898) from cases of suppurative otitis, by Hallé (1898) and Guillemot (1898) from cases of gangrene of the genital and pulmonary system respectively, and by Cottet (1899) from peri-urethral suppurations.

Meanwhile in Germany Krönig (1895) and Menge (1895) had independently described a strictly anaerobic streptococcus morphologically indistinguishable from *S. pyogenes*, which they found in the vagina in pregnancy, and later in collaboration (Krönig and Menge, 1897 ; Menge and Krönig, 1899) isolated several strains from the vagina and lochia in infected puerperal cases, as well as from parametrial suppurations and peritonitis. The observations of these workers served to demonstrate clearly the existence of streptococci of strictly anaerobic character, although they made little attempt to classify them beyond calling attention to

one group which produced gas and foul-smelling decomposition products in artificial media. Following upon the objections of Koblenck (1899) and others who still denied the existence of strictly anaerobic streptococci, Natvig (1905) confirmed the work of Krönig and Menge in describing six strains, all of which resembled the gas-producing group of these workers. From the similarity of his strains, Natvig concluded that all the anaerobic streptococci should be included in a single group, to which he gave the name *S. anaerobius* (Krönig). Lewkowicz (1901), however, had already isolated from the mouths of sucklings an extremely minute streptococcus growing only under strictly anaerobic conditions and producing neither gas nor odour in culture media. This organism, which he called *S. anaerobius micros*, was obviously very different from those already described by Veillon and Krönig, so that Natvig's conclusions were clearly unjustified.

From this time until 1910 strains of anaerobic streptococci isolated from various suppurative or gangrenous conditions were identified by their discoverers with the varieties named up to that time. In this year Schottmüller (1910[1]) described a strictly anaerobic streptococcus isolated chiefly from cases of puerperal sepsis, both from the local lesion and in some cases from the blood during life. This organism, which he compared with one of the strains described by Krönig and Menge, differed from *S. anaerobius* of Natvig in producing gas and odour only in media containing blood, remaining inactive in ordinary media. To it he applied the name *S. putridus*, believing that it determined the putrid character of the infection in many cases, although experimental inoculation of his strains failed to produce lesions in animals. Schottmüller (1911) and Rosowsky (1912) found *S. putridus* in the normal vagina, the latter in 40 per cent. of cases. Similar organisms were isolated by Hamm (1910), Warnekros (1911), Ozaki (1913) and others in various putrid infections, notably in cases of puerperal sepsis.

The literature of the War period contains few references to the anaerobic streptococci, attention at this time being drawn chiefly to the occurrence of anaerobic bacilli in septic wounds. Marwedel and Wehrsig (1915), however, described two cases of gas gangrene in wounds infected by anaerobic streptococci, in one of which the organism was grown in pure culture. Cottet (1918) found strictly anaerobic streptococci in 10 out of 33 war wounds associated with facultative anaerobes and other organisms, and Gerard and Romant (1919) isolated them in 7·5 per cent. of 133 cases, while Douglas, Fleming and Colebrook (1920) found them sufficiently frequently to justify anaerobic as well as aerobic culture in all cases.

More recently strictly anaerobic streptococci have been found amongst the normal vaginal flora by Schweitzer (1919), in lochia by Schmidt (1919), and in cases of purulent salpingitis by Curtis (1921). An organism generally identified as Schottmüller's *S. putridus* has been isolated from cases of pulmonary gangrene following puerperal sepsis (Bingold, 1921), aspiration broncho-pneumonia (Kissling, 1924), and septic tonsillitis

(Fraenkel, 1925). Brütt (1923) claims to have found it frequently in cases of suppurative appendicitis, and Lehmann (1926) describes three cases of acute endocarditis following septic abortion, where it was obtained from the blood in pure culture. Prévot (1924) has collected and described 13 strains of strictly anaerobic streptococci from 29 cases of pulmonary and puerperal sepsis. Of these he identifies 2 with *Micrococcus fœtidus*, 2 with *S. anaerobius* (Natvig), 3 with *S. putridus* and 2 with *S. anaerobius micros*. The remaining 4 strains differed from those previously described but presented only minor differences amongst themselves; for this reason Prévot has described them as a new species under the title of *S. intermedius*. Prévot's work is important because it represents the first serious attempt to separate the recognized anaerobic streptococci and to classify them according to their morphological and biological characters.

The present writer has succeeded in isolating 11 strains of strictly anaerobic streptococci, 2 from apparently normal frontal sinuses, 4 from cases of putrid pleurisy and pulmonary gangrene, and 5 from a series of 20 cases of dental sepsis. Thirty-one cases of suppurative mastoiditis and 25 of suppurative and gangrenous appendicitis have been examined and these failed to reveal a single instance. The following account of the anaerobic streptococci is based upon the writer's own observations and on information obtained from a survey of the existing literature.

Culture Appearances and Morphology.

Surface cultures of the anaerobic streptococci, of which only scanty descriptions are to be found in the literature, have been readily obtained by the routine use of a slight modification of the anaerobic jar of Fildes and McIntosh (1921). In this apparatus the organisms grow fairly readily on all the ordinary media, preferably those containing glucose, serum or blood. Growth is usually slow on first culture and the colonies are apt to be overrun by numerous organisms of other kinds, both aerobic and anaerobic, which are almost constantly present in the fresh pathological material; they may thus easily escape observation unless the medium is sufficiently lightly inoculated. Once isolated, their growth tends to improve on subculture. The appearances vary somewhat with different strains, but in general the colonies are smooth, flat and semi-transparent, with an even round margin, and frequently show a central raised papilla in older cultures. Individual colonies may reach a diameter of $1\frac{1}{2}$ to 2 mm. after 4 to 5 days. The colonies are non-viscid and emulsify readily. On chocolate agar a zone of reduction of characteristic pink colour appears round each colony, and this eventually spreads throughout the medium. The pink colour rapidly disappears on exposure to the air; although produced by other anaerobic bacteria besides the anaerobic streptococci, it has been found of assistance in separating these from the mixed growth usually obtained on first culture.

Since Veillon's first researches deep glucose-agar has been extensively employed for the cultivation of the anaerobic streptococci, although the

method does not lend itself to their rapid isolation from mixed growths. Growth occurs readily at incubator temperature, in the depth of the medium, with an upper limit situated at a varying distance (10 to 20 mm.) from the surface. The organisms form regular or conglomerate colonies of white or yellowish-grey colour, appearing finely granular under the low power of the microscope. These are visible to the naked eye after 48 hours, and attain their maximum diameter of rarely more than 1 mm. in 8 to 10 days. In this medium, with or without the addition of serum or fresh or heated blood, gas-formation, when it occurs, is readily observed. In deep shake cultures in chocolate agar, the surface of which is exposed to air, the reduction phenomenon described above is again noted. The medium below a certain depth is rapidly reduced to a pink colour, and, moreover, at the level which marks the upper limit of growth, a green ring makes its appearance in 24 to 48 hours. This green ring has been described by McLeod and Gordon (1923) in deep shake cultures of other anaerobes, and is presumed to be due to the formation of hydrogen peroxide. Once formed, the green product persists indefinitely.

The anaerobic streptococci grow readily under anaerobic conditions in ordinary fluid media provided that they have been boiled to expel their contained oxygen. Most strains produce a slight general turbidity with flocculent white deposit. They may, moreover, be grown aerobically in bouillon to which small pieces of liver or other parenchymatous organ have been added, since these substances remove oxygen from the medium and can maintain anaerobic conditions for several days (Wrzosek, 1907 ; Heim, 1910, &c.). Liver broth is therefore a suitable medium for the stock preservation of strains once isolated, subculture as a rule being necessary only once a week.

Morphology. The anaerobic streptococci show considerable morphological variation, though all stain well with the ordinary aniline dyes and are Gram-positive. The gas-forming group (*M. fœtidus*, *S. anaerobius*, *S. putridus*) closely resemble *S. pyogenes* in appearance ; they occur in diplococcal form and in short chains in pathological fluids and in growth on solid media, and with the exception of *M. fœtidus* tend to form longer chains of 20 to 30 cocci in fluid media. In the fresh material they are frequently associated with aerobic streptococci and a variety of other organisms, so that direct smears fail to give any indication of their presence. The individual members are 0·8 to 1μ in diameter and round or somewhat oval in shape. Capsules have not been demonstrated. Irregularities are frequently found in older cultures, where the cocci vary considerably in size and show occasional loss of Gram-staining. Of the non-gas-formers, *S. anaerobius micros* is characterized by its extremely minute size, individual cocci having a diameter of 0·25 to 0·4μ, while Prévot's *S. intermedius* (Prévot, 1925) is of intermediate size, having a mean diameter of 0·6μ. Five strains isolated by the writer from cases of dental sepsis were all of this intermediate size and tended to form short chains and irregular conglomerate masses on culture.

Biological Characters.

It is evident from the foregoing description that the anaerobic streptococci constitute a varied group differing radically from other streptococci in their intolerance to the oxygen of the air, and this fact adds greatly to the difficulty of their isolation and culture. The organisms are capable of growth only in media from which oxygen has been removed by the addition of reducing substances, or on the surface of media exposed to an atmosphere devoid of this gas. This property of strictly anaerobic growth appears to persist indefinitely ; the writer's own strains have shown no adaptability towards aerobic growth even after 50 generations or more. Mere traces of oxygen are sufficient to inhibit growth : in a series of partial pressure experiments in which different concentrations of oxygen were obtained by partial evacuation regulated by mercury manometer, growth never occurred if the oxygen tension exceeded 8 mm. of mercury, while most strains failed to yield surface growth if the oxygen tension exceeded 2 mm. In their anaerobic requirements these organisms are therefore comparable with *B. tetani*, which is recognized as one of the strictest of anaerobes. McLeod, employing a similar technique, found that *B. tetani* failed to grow at oxygen tensions greater than 2 mm. Hg, while cultures of *B. welchii* were still obtained at a tension of 30 mm.

The anaerobic streptococci grow best at body temperature in neutral or slightly alkaline media ; growth still occurs at 26°, but usually fails below 22° (although two of Natvig's strains showed growth at 20°). They are non-resistant to heat, half an hour at 60° or 10 minutes at 80° sufficing to kill them. Grown anaerobically in ordinary media they remain viable for several weeks ; one of the writer's strains was successfully subcultured from deep glucose-agar after six months. On the other hand, surface growths exposed to air at room temperature die out comparatively rapidly, and subcultures usually fail after two days of such exposure. Many strains produce gas and foul odour in culture. Veillon's (1893) original cultures of *M. fœtidus* invariably produced a foul odour, but gas formation was not constant. Natvig's strains of *S. anaerobius* usually produced both gas and foul odour ; in both varieties H_2S was absent from the gas formed. The *S. putridus* of Schottmüller formed no gas in ordinary media, but much gas containing H_2S in media to which blood or fresh tissue had been added. The other varieties—*S. micros* (Lewkowicz) and *S. intermedius* (Prévot)—produced neither gas nor odour in any medium.

Anaerobic streptococci are not hæmolytic and do not liquefy gelatin. Their fermentation reactions vary considerably even among different strains of the same organism ; the majority produce acid in glucose, maltose and lævulose, and some in addition ferment galactose and saccharose, while lactose, dulcitol, glycerin, inulin, and litmus milk usually remain unchanged. In view, however, of the variations observed, sugar fermentation tests are not likely to be of much assistance in their classification.

The agglutination reactions of the anaerobic streptococci as recently worked out by Prévot (1924) suggest that the group comprises a number of distinct but in some degree related members. He found that they were agglutinated by the homologous serum in dilutions of 1 : 25,000 (*S. anaerobius*), 1 : 5,000 (*S. putridus*), 1 : 1,000 (*S. intermedius*), 1 : 400 (*S. micros*), and 1 : 200 (*M. fœtidus*), while cross agglutination occurred at lower dilutions in some cases.

Pathogenicity.

The pathogenic power of the anaerobic streptococci when injected into laboratory animals is in general very small or non-existent. Most experimental inoculations have failed to produce more than slight lesions in rabbits, guinea-pigs and mice, even when the cocci are injected in relatively enormous doses. Occasionally local abscess formation has been observed and in some cases the organism has been recovered in pure culture from the contained pus, but spontaneous healing occurs within a few days. A solitary exception was seen in a fatal case of war-wound infection by Marwedel and Wehrsig (1915) where *S. putridus* was isolated in pure culture. This organism injected into a guinea-pig produced gas-gangrene and death within 12 hours. Natvig (1905) suggested that his failure to obtain experimental results was due to a rapid loss of virulence of the streptococci after isolation ; Marwedel and Wehrsig, indeed, found that their strain very rapidly became avirulent on subculture.

The negative experimental results reported by the vast majority of workers make it difficult to assess the importance of the anaerobic streptococci in human pathology. These organisms have been frequently found in normal subjects in the vagina and mucous cavities of the body, as well as in putrid or gangrenous conditions of the urino-genital tract, appendix, lungs and serous membranes. The question arises whether they are purely saprophytic, or whether they play a part in the production of the lesions from which they have been isolated. There is considerable evidence that, while primarily existing as saprophytes in the mucous cavities of the body, they may in favourable circumstances assume invasive properties, and alone or associated with other organisms may determine the special putrid character of the lesion concerned. Cases of mixed infection afford no certain criterion ; Schottmüller (1910), however, points out that in a number of cases of adnexal suppuration following puerperal sepsis *S. putridus* has been found in pure culture, and he regards this organism as a true parasite capable of giving rise in puerperal cases to a characteristic infection, namely, septic thrombophlebitis of the parametrial veins and abscess formation at the primary focus and in the lungs. Such cases, according to this worker, present a very typical clinical picture, including foul vaginal discharge, intermittent fever with rigors, anæmia, and secondary lung abscesses, while a positive blood culture is often obtained. Within recent years Schottmüller's views have been confirmed by a number of German workers, who have

found *S. putridus* in septic conditions of various kinds ; in pulmonary gangrene (Bingold, 1921[1], 1921[2] ; Kissling, 1924), gangrenous appendicitis (Brütt, 1923), septic tonsillitis (Fraenkel, 1925), and endocarditis following septic abortion (Lehmann, 1926). In many cases the organism has been cultivated from the blood-stream on repeated occasions during the course of the usually fatal illness. In spite of their invariable failure to produce experimental lesions with the strains isolated, these workers conclude from the clinical and pathological evidence that *S. putridus* plays an important part in the production of septic conditions, and in particular determines the putrid character of the lesions present. So far as the other varieties of anaerobic streptococci are concerned, no conclusions as to their pathogenicity are permissible in the present state of our knowledge.

Sensitiveness to Antiseptics : Chemotherapy.

By C. H. Browning.

Toward the antiseptics generally used, streptococci possess moderate susceptibility. In the case of *S. longus*, Lingelsheim (1912) obtained the following, among other, results :

Table VII.

Substance.	Concentration in Broth	
	Preventing Growth at 37° C.	Leading to Death in 15 Minutes at Room Temperature.
Mercuric chloride.. ..	1 : 65,000	1 : 1,500
Phenol	1 : 550	1 : 200
Malachite green	Less than 1 : 100,000	1 : 1,800

The alkalinity of the broth used in these tests corresponded to 5 to 7·5 c.cm. N/1 NaOH per litre.

Investigations on the antiseptic action of dyes have shown that streptococci are in general susceptible to basic compounds of the triphenylmethane series, e.g. methyl violet 6B (Jaenicke, 1890). Thus they fail to grow on agar containing gentian violet in a concentration of 1 : 100,000 (Churchman, 1912). Simon and Wood (1914) investigated for antiseptic properties a large number of dyes, which were incorporated in agar in a concentration of 1 : 100,000 ; a stroke inoculation was made on the medium, and the culture was kept at 37° C. for 48 hours and subcultures were then made. The majority of basic dyes, especially those of the triphenylmethane series, were found to be inhibitory for streptococci. But different strains of the organism varied in their susceptibility.

Also a strain which was originally resistant might later become susceptible. The actions of the dyes on streptococci and staphylococci did not always run parallel. According to Krumwiede and Pratt (1914), however, who tested a large number of strains, streptococci as a rule are more resistant to gentian violet and allied compounds, e.g. Hofmann's violet (dahlia), than are staphylococci, as 16 strains of the latter organisms failed to grow in a dilution of 1 : 500,000 of the dye. Schiemann and Baumgarten (1923) have made a similar observation in the case of dahlia ; but to brilliant green streptococci and staphylococci are about equal in their susceptibility according to the latter authors. The enterococcus is considerably more resistant to gentian violet (Churchman, 1912). In order to prevent growth of a strain of this organism in 0·7 per cent. peptone water, a concentration of 1 : 150,000 of brilliant green was required and 1 : 10,000 of the dye in ox-serum heated at 57° C. (Browning, Gulbransen, Kennaway and Thornton, 1917[1]).

The powerful antiseptic action of substances belonging to the diaminoacridine series, to which acriflavine belongs (see Browning, Gulbransen, Kennaway and Thornton[1 & 2], 1917 ; W. P. Morgan, 1918), has been extensively investigated by Morgenroth, Schnitzer and Rosenberg (1921), Gay and Morrison (1921), Weise (1923) and Schiemann and Baumgarten (1923), with a view to their use as chemotherapeutic agents. As tested on a number of hæmolytic strains isolated from human lesions, diaminoacridine methochloride (trypaflavine or acriflavine) prevented growth on the average in a concentration varying from 1 : 1,000,000 to 1 : 100,000 in peptone water medium and in serum, whereas a concentration at least four times greater was required in parallel tests with ethoxy-diaminoacridine hydrochloride, i.e. rivanol (Browning and Gulbransen, 1928 ; see also Weise, 1923 ; Laqueur, Sluyters and Wolff, 1924). According to Gay and Morrison (1921) acriflavine is more actively antiseptic toward streptococci in pus than in broth ; but this is probably an exceptional result, since it has been generally found that in the presence of pus the antiseptic action is diminished (see Eggerth, 1926). Other things being equal, the hydrogen-ion concentration plays an important part, bactericidal action of the aminoacridine compounds being intensified when the pH of the medium is increased (see Browning, Gulbransen and Kennaway, 1919 ; Fleischer and Amster, 1923 ; Eggerth, 1926), although Michaelis and Hayashi (1923), while confirming this finding with rivanol, could not do so for trypaflavine.

Proceeding from the observation that ethyl-hydrocupreine (optoquine) was highly antiseptic towards pneumococci the action of substances belonging to the quinine series has been investigated by Morgenroth and Tugendreich (1917), who tested eight different strains of hæmolytic streptococci, most of which were freshly isolated from human infections. The method adopted was to determine the concentrations of the various substances which (*a*) caused death of the organisms, and (*b*) permitted growth, when they were incorporated in ascites-broth ; 2 c.cm. of medium

containing the antiseptic were inoculated with 2 drops of a 24-hours' culture in ascites-broth, and then the mixtures were kept at 37° C. for 24 hours. Blood-agar plates were inoculated with the mixtures at the time of inoculation and again after the period of incubation. Since growth occurred in the former it was clear that the small quantity of the antiseptic substance carried over into the subculture did not prevent growth. The later cultures indicated the lethal concentrations of the compounds. In addition, 0·5 c.cm. of the incubated mixtures were injected intraperitoneally into mice in order to determine whether alterations in virulence of the organisms had occurred as a result of the action of the antiseptic. The following results were obtained: the lethal concentration of quinine hydrochloride and of hydroquinine lay between 1 : 1,000 and 1 : 4,000, the latter concentration permitting vigorous growth. In the hydrocupreine series the ethyl compound (optoquine) did not differ markedly from quinine. Schiemann and Ishiwara (1914) had previously found that streptococci differed from pneumococci in being relatively insusceptible to optoquine and this has been proposed by Nachmann (1916) as a method of differentiating the two organisms.

With higher members of the series the antiseptic power was greatly increased, the lethal concentration of isoamyl hydrocupreine bihydrochloride (eucupine) lying between 1 : 16,000 and 1 : 32,000 and that of iso-octyl hydrocupreine bihydrochloride (vuzine) between 1 : 64,000 and 1 : 128,000. The last substance was the most lethal of the series towards streptococci, since higher homologues showed a falling off in activity—the bihydrochlorides of decyl- and dodecyl-hydrocupreine having respectively only one-quarter and one-eighth of the potency of vuzine. Those substances in general acted slowly, the concentration required to kill the organisms in 2 hours being considerably higher than that which was lethal in 24 hours. The derivatives of eucupine and vuzine analogous to quinotoxine, eucupinotoxine and vuzinotoxine are, however, specially characterized by rapidity of lethal action (Morgenroth and Bumke, 1918). This is attributed on theoretical grounds to lack of capacity of the organisms to alter their resistance to the latter substances by 'chemoflexion'. The intensifying effect on the bactericidal action of the quinine series brought about by increase in the pH of the medium is interpreted by Michaelis and Dernby (1922) as indicating most probably that the action is due to the free bases and not to the salts. The antiseptic action of vuzine is markedly diminished by the presence of serum (Morgenroth, 1919).

Some variation was met with in the resistance of different strains to substances of the hydrocupreine series; further, cultures which had been kept on artificial media for some months became more resistant. Non-hæmolytic streptococci (*S. viridans*) also possess relatively high resistance, e.g., the lethal concentration of vuzine being for these between 1 : 10,000 and 1 : 20,000 (see also Koch, 1920). A striking observation was that in the case of those substances low concentrations which permitted vigorous growth of virulent cultures of streptococci frequently led to loss

of their pathogenicity for mice. Similarly, Nakamura (1924) has shown that streptococci which had been acted upon by trypaflavine for a short period, e.g., a concentration of 1 : 25,000 of the dye acting for 15 minutes, although not completely killed, were nevertheless so reduced in virulence as to be unable to cause infection in mice when inoculated into a recent wound. A 1 : 12,500 solution of mercuric chloride required to act for 1 hour on the organisms in order to reduce their virulence to the same extent. These results differ from those of Rodewald (1923), who found that death, as tested by sterility on subculturing, resulted before loss of pathogenicity; but his methods involved treatment of very dense suspensions of organisms and subsequent washing.

Hæmolytic streptococci, unlike staphylococci, are very susceptible to the antiseptic action of soaps, especially laurate, oleate, linoleate and linolenate of sodium or potassium, although they are considerably more resistant than pneumococci (Walker, 1924). Thus, a N/640 watery solution (0·035 per cent.) of sodium laurate was lethal for streptococci in 15 minutes—1 per cent. of phenol was required to kill the organisms in the same time. Serum greatly lowered the effect, e.g. in the presence of 5 per cent. serum, N/80 sodium laurate was required to kill streptococci in 15 minutes, i.e. 8 times the lethal concentration in water. Larson and Nelson (1925) found sodium ricinoleate actively bactericidal toward *S. scarlatinæ*. Eggerth (1926) found that acriflavine and soaps, especially oleates, intensified each other's antiseptic action on streptococci. According to Lemay and Jaloustre (1924) streptococci are markedly resistant to the antiseptic action of soluble bismuth compounds to which *Staphylococcus aureus* is highly susceptible.

It has not been found possible to render virulent streptococci resistant to antiseptics (Morgenroth, 1924). In the case of streptococci of *viridans* type which may be isolated a short time after the injection of hæmolytic streptococci into an animal, and which are apparently derived from the latter, Morgenroth and Schnitzer (1923[2&3]) observed as a rule great diminution in susceptibility to rivanol accompanied by marked reduction in virulence, whereas the parent strains were highly susceptible. On the contrary the susceptibility to vuzine of the *viridans* organisms thus obtained was little or not at all reduced. Again, hæmolytic streptococci recovered by culturing from the subcutaneous tissue of mice after local inoculations of *S. viridans* (derived from cases of endocarditis) followed by injections of rivanol were found to be relatively resistant to rivanol (Freund, 1923). On the other hand, hypersensitiveness to trypaflavine resulted when streptococci were grown in fluid media containing high dilutions of the antiseptic, e.g. 1 : 10,000,000 for 24 hours; a similar effect was obtained also when mice were both inoculated and treated intraperitoneally with this compound, and then the organisms were recovered from the blood of the tail-vein 24 hours later (Schnabel and Kasarnowsky, 1924). The organisms so obtained were not hypersensitive to optoquine or mercuric chloride.

Arsenical compounds of the salvarsan group behave *in vitro* as powerful, but slowly acting, antiseptics towards hæmolytic streptococci (Allison, 1918; Colebrook, 1928). Thus in some of Colebrook's experiments salvarsan was lethal in 24 hours in a concentration of 1 : 10,000 in serum. Streptococci of *viridans* type and especially enterococci showed greater resistance. The antiseptic property may be conferred on the serum in the human subject by an intravenous injection of the drug; it is most marked in serum withdrawn immediately after the injection and diminishes at first rapidly then more slowly during 24 to 48 hours. But by intramuscular or subcutaneous injections of suitable compounds at appropriate intervals the antiseptic property of the serum may be maintained for several days or even weeks. Colebrook has also found that in general the harmful action *in vitro* of these arsenical drugs toward hæmolytic streptococci is greater than toward human leucocytes. Correspondingly, the bactericidal and inhibitory action on streptococci of defibrinated whole blood was considerably increased in several cases after administering arsenical compounds to the human subject.

Successful chemotherapeutic intervention in local streptococcal infections was effected originally by Morgenroth and Abraham (1920). They proceeded from the observation that cultures of hæmolytic streptococci recently isolated from pyogenic infections in the human subject when injected in suitable doses into the subcutaneous tissue of the mouse, cause a local area of suppuration, a ' phlegmon '. Accordingly, 0·1 c.cm. of such an inoculum was injected under the abdominal skin, and this was immediately followed by the injection as far as possible in the same situation of 1 c.cm. of a solution of the substance to be tested. The result was usually determined by killing the animal 24 hours later, and making a culture from the subcutaneous tissue at the site of inoculation. In the case of vuzine, on the average, an injection of 1 : 2,000 solution led to sterile cultures. Control experiments showed that when a 1 : 100 solution was injected subcutaneously after inoculation and the animal was immediately killed and cultures made on blood-agar, an abundant growth of streptococci occurred. Accordingly, in the therapeutic tests the negative results were not due to carrying over of the chemical substance on to the culture medium. As regards the rate of destruction of the organisms, it was found that two hours after treatment with a 1 : 800 solution the subcutaneous tissue was sterile. Eucupine was nearly as active as vuzine; but with quinine, hydroquinine or optoquine concentrations 40 to 50 times greater were required, which were often toxic for the treated animals. Fresh solutions of vuzine were essential, since they became less active on standing without apparently undergoing any other ascertainable physical or chemical change. Vuzine in addition to its high antiseptic potency was the member of the series specially suited for such local action, or ' deep antisepsis ', on account of its not diffusing rapidly from the site of injection. For, as compared with optoquine, according to Morgenroth's (1919) observations, vuzine becomes fixed by the tissues

locally, but is not thereby inactivated. This handing over of the antiseptic from tissue elements to micro-organisms, which are thereafter killed, has been termed 'transgression' (see also Schnitzer and Berger, 1923). Schiemann (1923) had found that the life of mice could be preserved after infection with streptococci by injecting trypaflavine (acriflavine) intraperitoneally 5 to 30 minutes after inoculation by the same route.

Morgenroth, Schnitzer and Rosenberg (1921) investigated the therapeutic action of a series of acridine derivatives in subcutaneous infections of the mouse. According to their observations there was a lack of correspondence between the results *in vitro* and *in vivo*; thus of two substances which were practically equal in their action on a given strain of streptococci in the test-tube, one might be greatly inferior to the other as tested by their action on subcutaneous infections in mice. Further, of two substances, the one which was inferior *in vitro* might be markedly superior in the animal test. An additional difficulty was that substances of this series which acted well on infections with a particular strain of streptococci often failed when tested with other strains.

The compound 'rivanol' (ethoxy-diaminoacridine), however, was stated to act well *in vivo* on practically all strains—'pantherapeutic action'—since on the average a concentration of 1 : 40,000, when used as above described, sterilized the subcutaneous tissue of infected mice (see also Schnitzer and Rosenberg, 1924). When there was an interval between inoculation and treatment the latter was also frequently successful (Morgenroth and Schnitzer[1], 1923), e.g. cure was effected by injecting at the site of inoculation after about 4 to 6 hours either 1 c.cm. of a 1 : 2,000 solution of rivanol or 3 c.cm. 1 : 500 vuzine or in the case of the latter substance 3 c.cm. of a 1 : 1,000 solution followed by a similar amount of a 1 : 2,000 solution on each of the two following days. Sterilization was effected in almost every case with rivanol when treatment was begun 18 hours after inoculation and consisted, e.g., of two injections each of 3 c.cm. 1 : 5,000 to 1 : 8,000 solution given at an interval of 24 hours (confirmed by Doskocil, 1925, in the case of not too virulent infections). In addition to the local sterilization the blood was also sterile when the animals were examined 5 days later. A comparison of the rates at which streptococci are killed by rivanol *in vitro* and in the subcutaneous tissue of the mouse has shown that in the latter case the process is more rapid and has practically reached its limit in 4 hours (Amster and Rother, 1924). But according to Schnitzer and Munter (1924), commonly the action progresses further, and when cultures are made from the subcutaneous tissue after 24 and 72 hours it is found that a lower concentration of the drug has been effective after the longer than after the shorter period. The reliability of the subcutaneous streptococcal phlegmon in the mouse as a test object for determining quantitatively the effectiveness of chemotherapeutic agents has been doubted, however, by Braun and Goldschmidt (1927). They found that often the pyogenic reaction soon spontaneously ceases to progress. Laskownicki (1924) repeated the experiments of Morgenroth and his

co-workers, using for treatment 0·5 c.cm. of 1 : 500 to 1 : 5,000 solutions of rivanol; but very little curative action was apparent. Also Browning and Gulbransen (1928) obtained irregular results when comparing the action of rivanol with acriflavine, both by this method and a modification in which the antiseptic solution and the organisms were injected as a mixture. Accordingly they concluded that the method did not permit of a satisfactory comparison of the efficacy of different chemotherapeutic substances.

It has been shown that in mice infection with virulent streptococci introduced into recent wounds may be cured by subsequent applications of antiseptics, especially trypaflavine (acriflavine), even when the interval between inoculation and a single brief application of the antiseptic is as long as $\frac{1}{2}$ to 1 hour (Reinhardt, 1922; Schiemann and Wreschner, 1922). These results were confirmed by Weise (1923), who found also that vuzine 1 : 500 was much less effective than acridine compounds, trypaflavine 1 : 500 or rivanol 1 : 100 (see also Collier and Bernhagen, 1928). Excision of the surface of the wound $\frac{1}{4}$ to 2 hours after inoculation had no effect in saving the life of the animals (Weise). Treatment was as effective in animals inoculated with streptococcus-containing tissues or blood obtained directly from other animals, as with broth cultures. Infections in deep wounds of muscles were less amenable to treatment than those of superficial wounds.

Treatment of an infected wound may not prevent the organisms from generalizing, but the infection may remain latent until lighted up by some other condition such as an intercurrent infection. Thus Reinhardt (1922) records that mice in which a wound infection had been cured by the application of trypaflavine half an hour after inoculation were inoculated 11 days later with diphtheria bacilli. When they died after 5 to 8 days, streptococci were found in the blood, spleen and peritoneal cavity.

Sanocrysin (sodium gold thiosulphate) and other gold-containing compounds were tested by Schiemann and Feldt (1926). A large proportion of mice were cured by sanocrysin administered subcutaneously at once or even several hours after intraperitoneal inoculation with a multiple of the lethal dose of a culture isolated from a case of puerperal fever; intravenous treatment was not so effective. The infection with a more highly virulent strain (Aronson's), however, was not influenced even when the therapeutic agent was injected intraperitoneally. When the inoculation was made by rubbing cultures of the virulent strain into a skin wound, sanocrysin injected subcutaneously (1 c.cm. 1 : 200 to 1 : 500 per 20 gm. mouse) up to one hour later at a site remote from the wound cured the animals. Rivanol (1 : 1,000) or trypaflavine (1 : 2,000) administered in this way had little action. On the other hand, the latter substances when brought directly into contact with the infected wound were more effective than sanocrysin similarly applied.

Gay and Morrison (1921) attempted the treatment of experimental streptococcus empyema in rabbits produced by injecting a culture of a

passage strain into one pleural sac. In untreated animals this produced a progressive infection which spread through the pericardium to the other side and caused death in from 3 to 7 days ; septicæmia was present only in the terminal stages. Treatment was begun some hours up to several days after inoculation, acriflavine being used in the majority of the experiments and one or several injections being given ; in addition, fluid which accumulated in the pleural sacs was aspirated. These authors conclude that, although they nearly sterilized the pleural sacs, regrowth of the organisms always occurred, and in no instance was the life of the animal prolonged. Combined treatment with acriflavine and anti-streptococcus serum was not more successful. Eggerth (1926) employed also mixtures of proflavine (diaminoacridine salt) and sodium oleate with similar results.

Allison (1918) attempted to treat streptococcal infections in rabbits by intravenous injections of salvarsan compounds. The effects of such treatment were shown by the fact that the blood was usually rendered sterile and a high leucocytosis was maintained, whereas in untreated animals the leucocytes fluctuated, as also did the content of the blood in organisms. A definite curative effect was not obtained, however, owing to the occurrence in the more chronic infections of local lesions, e.g. arthritis, which probably acted as reservoirs of the organisms, while in very acute infections toxic action caused the serious effects.

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CHAPTER III. THE PNEUMOCOCCUS.

(Synonyms : *Streptococcus lanceolatus, Diplococcus pneumoniæ.*)

By W. Mair (Metropolitan Asylums Board), J. W. McLeod (University of Leeds), F. Griffith (Ministry of Health); with Sections by C. H. Browning (University of Glasgow) and W. Bulloch (London Hospital).

Introduction.

By W. Mair.

The streptococcus with which this chapter deals is characterized by the presence of an active autolytic ferment. This character marks it off clearly from other streptococci, and there is no other bacterial species in which the phenomenon of autolysis is so pronounced and so constant. A comparison with the transmissible lysis described by Twort and d'Herelle at once suggests itself, but since all known strains are subject to autolysis it is not possible to demonstrate an agent capable of initiating transmissible lysis. The property of dissolving in bile is, in all probability, another manifestation of the same character.

A capsulated diplococcus causing septicæmia in the rabbit was first described by Pasteur (1881) as occurring in the saliva of a child suffering from rabies. Fraenkel (1886) found the same diplococcus in pneumonic sputum, and Weichselbaum (1886) established its importance as a cause of pneumonia. The latter drew as clear a distinction as was then possible between his *Diplococcus pneumoniæ* (i.e. pneumococcus), which he found in 72 per cent. of his series of 129 cases of pneumonia, and his *Streptococcus pneumoniæ*, which occurred in 16 per cent. This distinction seems to have been lost sight of by subsequent writers and there has been a tendency to regard all diplococci found in pneumonic lung as pneumococci without due regard to differences in their character.

Schottmüller (1903), using human blood-agar plates for the differentiation of streptococci, described a variety to which he gave the name *Streptococcus mucosus*. The colonies in question were characterized by a watery mucoid appearance with green discoloration of the medium. After two days' incubation the mucoid character disappeared, and no hæmolysis was observed until after several days. This streptococcus is now known to be a variety of pneumococcus. Although it can, as a rule, be distinguished from other pneumococci by its morphology and its appearance in culture, it is closely similar in its pathogenic action and chemical and biological characters (see Chapter II). The various strains

of this *mucosus* variety of pneumococcus belong for the most part to one serological type, viz. Type III of the American classification.

The importance of the pneumococcus as a pathogenic micro-organism, its wide distribution in the respiratory passages, not always apparently in association with disease, and the peculiarities of its behaviour in artificial culture have led to a very intensive study of the micro-organism. To American investigators in particular we owe our present knowledge of the conditions of growth, serological varieties, and biochemistry of the pneumococcus, while the work of Heidelberger and Avery on the chemical constitution of the substances which determine the serological specificity of the different types has opened the way to a better understanding of the chemistry of immunity.

Morphology.

By W. Mair.

The pneumococcus is an oval or oat-shaped diplococcus. The arrangement in pairs is best seen in body fluids or in culture media containing serum. The members of a pair are sometimes pointed only at the distal ends, giving the lancet shape to which the organism owes its systematic name, *Streptococcus lanceolatus*. Marked variation in shape and size may be observed in the same specimen. Round forms are common, occurring either singly or in pairs or in short chains. In cultures, especially in fluid media, the tendency to chain formation is more marked than in the animal body, and round forms are predominant.

The pneumococcus stains easily with any of the basic dyes and retains the stain in Gram's method. Cultures, even when quite young, often show Gram-negative forms, and this loss of the characteristic staining property is probably associated with the beginning of autolysis.

The formation of a capsule is one of the most prominent features of the pneumococcus. The capsule reaches its fullest development in the animal tissues. In cultures it is more difficult to demonstrate, except in the *mucosus* variety. In the body fluids the capsule is seen clearly defined by a sharp outer line ; in fluid cultures its outline is often hazy, and the cocci appear to be surrounded by a substance which is passing into solution. A single oval capsule surrounds the lancet-shaped diplococcus ; when each individual in a pair is oat-shaped the common capsule is indented in the middle. The size of the capsules varies much in different strains and in the same strain under different conditions. In the *mucosus* variety the capsule is particularly well marked, in cultures as well as in animal tissues. This variety is further distinguished by the round form of the cocci and a greater tendency to grow in chains on solid media. The capsule may be demonstrated by staining by Gram's method and counter-staining with eosin after fixation in strong formalin (Wadsworth, 1906) or 10 per cent. tannic acid (Rosenow, 1911[1]). Hiss's (1902) method is simple and reliable, and that of Richard Muir gives a beautiful picture when successful. The capsule can also be demonstrated by the Indian-ink method of Burri.

Cultural Characters.

By W. Mair.

The pneumococcus grows on ordinary simple media, though not readily. For the development of its distinguishing characteristics, and, in particular, for the maintenance of its virulence special requirements must be fulfilled.

Growth occurs under aerobic and anaerobic conditions, but with some strains it is difficult to obtain anaerobic growth. The optimum temperature for growth is 36 to 37° C. No growth occurs below 25° C. or above 40° C.

Growth on nutrient agar. On nutrient agar small, round clear colonies are formed, which are not easily differentiated from those of other streptococci.

Growth in broth. In broth growth occurs in the form of a slightly turbid uniform suspension with a small disc-like deposit at the bottom of the tube. In cultures which have lost their virulence the growth may settle to the bottom, leaving the supernatant fluid clear, but the culture is easily shaken up to form a uniform suspension. On further incubation after the maximum growth has been reached, the culture gradually becomes clear owing to autolysis. It is usual to find a broth culture after two days' incubation quite as clear as before inoculation. If, however, the culture becomes acid owing to the presence of glucose autolysis may not occur. The reaction of the medium must be carefully adjusted. The optimum is pH 7·8, and the limits between which growth can be obtained are pH 7·0 to pH 8·3. The initiation of growth in fluid media depends in a marked degree on the size of the inoculum (Gillespie, 1913), and the period of lag is largely influenced by the age of the culture used for the inoculation (Chesney, 1916).

In order to obtain more abundant growth various substances are added to the ordinary media. Of these the most useful are serum and blood, the presence of which obviates the necessity for the finer adjustment of the reaction. The addition of glucose also favours growth, but, unless special precautions are taken, the resulting acidity soon inhibits it and the cultures die out rapidly. Hiss (1905) added calcium carbonate to keep the reaction of the medium nearly neutral; this method has obvious disadvantages, and the same object can be attained by restricting the amount of glucose to 0·1 per cent. and adding phosphate to act as 'buffer'. The concentration of phosphate should not exceed 0·1 molecular, and no other salt should be added, as larger amounts of salts have an inhibitory action (Dernby and Avery, 1918). In broth made from meat infusion and peptone, 5 gm. per litre of air-dried sodium phosphate may be added instead of the usual sodium chloride. With tryptic digest broth, made according to the directions of Douglas and Hartley (1922), phosphoric acid should be used for neutralization after the digestion.

Growth on serum-agar. On serum-agar slopes or on the surface of plates the growth of pneumococcus resembles generally that of other streptococci. The colonies are rather more transparent. Not infrequently

they show a ringed appearance with a depressed and more transparent centre. This appearance is due to autolysis of the central and older part of the colony. Well-separated colonies may grow to a considerable size, 2 to 3 mm. in diameter. These on further incubation become less conspicuous and may almost disappear. If incubation is still further prolonged a new growth in the form of white papillæ may be observed.

The *mucosus* variety shows large transparent, watery colonies, well raised at first above the surface and apt to form confluent streaks. On further incubation these dry down so as to become almost invisible.

Growth on blood-agar. On agar plates made with the addition of 2 to 5 per cent. of fresh blood, surface colonies of pneumococcus, when typical, show as smooth, slightly raised, flat discs of variable size. Viewed by reflected light they are whitish or almost colourless ; by transmitted light they look more or less green. The green colour is due to a discoloration of the red blood corpuscles underlying the colony. The discoloration may extend beyond the colony, and as it is accompanied by a partial hæmolysis there results a lighter green surrounding zone. If the plates after incubation are placed in the ice-chest over-night a zone of complete hæmolysis is found outside the discoloured zone. Apparently, the discoloured corpuscles are insensitive to hæmolysis. Under anaerobic conditions hæmolysis, if it occurs at all, is of the complete β type, and there is no green discoloration (Brown, 1919).

It is usually possible to distinguish pneumococcal colonies from those of other streptococci of the *viridans* group by their larger size, soft consistence and smooth surface ; sometimes also the colonies are ringed as on serum-agar. The growth of the *mucosus* variety shows the mucoid type of colony already described, and the underlying medium shows the usual discoloration and partial hæmolysis.

On heated blood-agar, pneumococcus colonies are yellow in colour, like those of other streptococci of the *viridans* group, and are not so easily differentiated as on the unaltered medium.

Growth in blood-broth. Growth in blood-broth is indicated by a purple discoloration of the blood, which later becomes dark brown. This colour change is due to the formation of methæmoglobin. The altered corpuscles are protected from hæmolysis as on the blood-plates. The medium is useful for the preservation of stock cultures.

Viability. Cultures of pneumococcus, especially in fluid media, die rapidly if kept in the incubator or at room temperature. On solid media they survive for considerable periods if kept in the ice-chest, as also in well-sealed tubes in the incubator at 37°. Pneumococci in animal fluids survive for long periods if rapidly and thoroughly dried (Heim, 1905). Cultures desiccated at a low temperature by the method of Swift (1921) also remain viable and retain their virulence for long periods. Whether in culture or in exudates, heating to 60° C. for half an hour is sufficient to kill pneumococci.

Autolysis and Solubility in Bile.

By W. Mair.

Neufeld (1900), making observations with pneumococcus on the lines of Koch's work on immunization against 'rinderpest', found that bile has a specific bacteriolytic effect on pneumococcus; this observation is the basis of the bile-solubility test for distinguishing pneumococcus from other streptococci of the *viridans* group. Neufeld, however, originally maintained that it is only freshly isolated and virulent strains which show this reaction, and although Levy (1907) proposed to use the test as a definite means of differentiation, many writers, influenced apparently by Neufeld's view, have been willing to accept as pneumococci strains which do not dissolve in bile and which do not readily autolyse. This attitude has led to unnecessary confusion. It is true that different strains of pneumococcus show varying sensitiveness to the action of bile just as they vary in the readiness with which they undergo autolysis in culture, but with a satisfactory technique one is very seldom in doubt as to whether a particular strain should be classed as bile-soluble or not, and strains which have been kept on culture media for long periods retain the property.

Technique of the bile-solubility test. Different samples of bile naturally vary in their activity. A satisfactory specimen of ox-bile should dissolve a broth culture of pneumococcus when added in a dilution of 1 in 10. After standing ten minutes the tube should be as clear as it was before inoculation. The bile may be sterilized in the autoclave and kept indefinitely. The substances in bile most actively concerned in the reaction are probably the salts of the conjugated cholic and desoxycholic acids. If the unconjugated acids in a pure state are tested it is found that the sodium salt of desoxycholic (or choleic) acid is ten times as effective as that of cholic acid. It is, therefore, best to use a solution of pure sodium desoxycholate for the test. A 10 per cent. solution of this salt keeps indefinitely, and for the test 0·1 c.cm. of this solution should be added to 5 c.cm. of broth culture or suspension of pneumococcus, giving a concentration of 1 in 500 of the salt. The culture must not be more acid than pH 6·6, as at this point precipitation of the bile-acid begins. At pH 6·8 the bile-acid separates slowly in the form of a jelly after the culture has cleared. With most strains of pneumococcus the culture clears up completely in a few minutes even at a dilution of 1 in 1,000, and if, with a dilution of 1 in 500, complete clearing does not occur after 1 hour at 37° C., the streptococcus in question should not be regarded as a pneumococcus. It need hardly be pointed out that with a mixed culture partial clearing may occur. For the purpose of the test it is most satisfactory to grow the organism in 0·1 per cent. glucose phosphate broth of reaction pH 7·8, and phenol red in a concentration of 1 in 110,000 may with advantage be added. This does not interfere with the growth of bacteria, and the colour change indicates fairly accurately the H ion concentration, making it easy to adjust this, if necessary, by the addition of alkali.

This indicator broth is also useful in the study of pneumococcus when, as sometimes happens, autolysis starts very early. The opacity due to growth may then be missed, and the only indication that growth has occurred is the colour change.

Although it is sometimes stated that serum prevents the solvent action of bile on the pneumococcus, the test can be carried out equally well in serum-broth cultures.

A method of preparing desoxycholic acid from bile has been described by Mair (1917). The use of commercial ' sodium taurocholate ' in place of bile is not to be recommended. Its activity varies as much as that of bile, and it has the further disadvantage that a precipitate, which is apt to obscure the reaction, slowly forms on its addition to broth cultures.

Limiting conditions for autolysis and bile-solubility. The conditions necessary for the autolysis of the pneumococcus and for bile-solubility are, so far as is known, identical. There is every reason to believe, therefore, that the bile-salt acts simply by hastening the natural autolytic processes. It is of interest to note in this connection that some growth, with early autolysis, may occur in broth containing sodium desoxycholate in a strength of 1 in 2,500, while a culture of the same strain clears up completely within an hour when the same amount of the bile-salt is added to it at its maximum opacity. The rate at which autolysis takes place depends on the H ion concentration of the culture when growth ceases and on the temperature to which the culture is subsequently exposed. The following observations were made with broth cultures of varying H ion concentration. The acid range was obtained by increasing the amount of glucose in the cultures ; the alkaline range by adding sodium hydrate to the grown cultures. For the corresponding observations on bile-solubility sodium desoxycholate was added to the cultures in a strength of 1 in 1,000.

The range of H ion concentration within which autolysis occurs is from pH 8·5 to pH 6·0. The optimum is pH 6·8. For bile-solubility the acid end of the range is restricted, since the bile-acid is thrown out of solution at pH 6·5. The alkaline limit is the same as for autolysis.

Lord and Nye (1922) obtained somewhat different figures for the range of H ion concentration within which autolysis occurs. By suspending washed living pneumococci in phosphate solutions of varying reaction, they found that autolysis took place most rapidly between pH 7·0 and pH 5·5. They did not observe the optimum H ion concentration at pH 6·8, and did not determine the extent of the alkaline range.

Autolysis takes place at temperatures from 5 to 50° C. Its rate increases with rise of temperature to a maximum at 42° C. This maximum is due to partial destruction of the autolytic ferment at higher temperatures. The autolysin is more sensitive to heat when the reaction is alkaline.

Heating at 50° C. for half an hour at pH 8·2 prevents subsequent autolysis at 42° C., and at 22° C. on the addition of bile-salt no immediate clearing takes place, but after 24 hours at room temperature clearing occurs. Heating at 50° C. for half an hour at pH 7·8 prevents subsequent

autolysis at 42° C., and delays but does not prevent autolysis at 22° C. or bile-salt lysis. Heating a culture at 56° C. for half an hour (pH 7·0 to 6·8) prevents autolysis and bile-solubility. This is due in both cases to a destruction of autolysin, for if the centrifuged deposit from a heated culture be suspended in an autolysed culture the heated pneumococci undergo autolysis which can be hastened in the usual manner by the addition of bile-salt. It will be noted that the heat required to destroy the autolysin is also that necessary to kill the culture, and is less than that required for the destruction of the lytic agent in bacteriophage phenomena.

If the deposit from a culture in glucose broth which has become acid (pH 5·0), and has in consequence died, is washed and resuspended in phosphate solution of pH 6·8, autolysis takes place so rapidly that it is somewhat difficult to demonstrate the usual action of bile-salt in hastening the process. This is possibly the explanation of the statement sometimes made that such pneumococci are no longer bile-soluble. Here the action of the autolysin has been inhibited by the acid produced in the culture and at the same time the cocci have been damaged, so that when a suitable reaction is restored they are unusually sensitive to the autolysin.

Pneumococci may be rendered more sensitive to the action of their autolysin by other reagents, although in no other case is the reaction so dramatic as with bile-salts. Thus, if a culture be shaken up with chloroform and placed in the incubator, autolysis takes place within a few hours. Sodium oleate (Lamar, 1911) and salts of other unsaturated fatty acids found in bile (Kozlowski, 1925) have a similar action. It is not possible to demonstrate a similar rapid effect with alcohol, ether, acetone or hydrogen peroxide, but it is probable that any reagent which damages the pneumococcal cell without destroying the autolytic ferment must hasten autolysis.

Atkin (1926) has made the interesting observation that the papillæ which appear on the site of autolysed colonies on prolonged incubation of serum-agar cultures are either devoid of autolysin or resistant to its action, and suspensions made from them are not bile-soluble. Subcultures from these papillæ, however, are bile-soluble and undergo autolysis as usual.

In their studies on the intracellular enzymes of pneumococcus, Avery and Cullen (1923) have shown that solutions of washed pneumococci contain a ferment which has a bacteriolytic effect on pneumococci which have been killed by heating to 60° C. for half an hour, or have been autoclaved at 120° C. for 20 minutes. The optimum H ion concentration for the action of this enzyme lies between pH 6 and pH 8. Its activity is destroyed by heating at 60° C. for half an hour. It has no effect on β hæmolytic streptococci or *Staphylococcus aureus*, but is stated to have a much slower bacteriolytic effect on other streptococci of the *viridans* group. There can be little doubt that this bacteriolytic enzyme is identical with the autolysin. Avery and Cullen state that the bacteriolytic enzyme has no action on living pneumococci. It is difficult to see how this could

be definitely determined, but *a priori* it seems unlikely that living and undamaged cells could be sensitive to their own autolysin. If the bacterial extract is added in the same amount to two cultures of the same age and opacity, one of which has been heated to 56° for 20 minutes, the heated culture clears sooner than the living one, showing that the dead bacteria are more sensitive to the autolysin than the living, which is what one would expect.

Biochemistry.

By W. Mair.

Our knowledge of the chemical changes which take place in cultures of pneumococcus is still very far from complete. For the most part these are indicated only by biological reactions which the culture fluid acquires. The readiness with which autolysis occurs makes it difficult to distinguish between extracellular and intracellular processes, and no line can be distinctly drawn between the results of the metabolic processes of the living bacteria and those of the disintegration of their cellular substance. Many of the metabolic activities of the living culture can be reproduced by sterile autolysates of the pneumococcal cells, which are thus shown to contain various enzymes.

Proteolytic Action.

Proteoses and peptones are hydrolysed, as shown by increase of amino-nitrogen. There is evidence of proteolytic action on some intact proteins (see p. 174), although no liquefaction of gelatin or of inspissated serum occurs. Platt (1927[1]) has shown that the addition of 3 per cent. of gelatin to peptone-water renders this more suitable for the growth of pneumococcus, and also that growth occurs in a solution of gelatin which has been autoclaved for 20 hours. He concludes that relatively complex protein decomposition products are required for the growth of pneumococcus.

Fermentation of Carbohydrates, Alcohols, &c.

Pneumococci ferment actively with the formation of lactic acid, the *mono-saccharides* glucose, mannose, galactose and lævulose; the *di-saccharides* lactose, maltose and saccharose; the *tri-saccharides* raffinose and trehalose; the *polysaccharides* inulin and dextrin and the *glucoside* salicin. The acid death-point in carbohydrate media is at pH 5·0. This degree of acidity may occur even in phosphate broth when the content of glucose exceeds 0·3 per cent. (Avery and Cullen, 1919; Lord and Nye, 1919). The fermentation of *alcohols* is much less marked. Slow acid production takes place in media containing glycol, glycerol and erythritol; some strains slowly produce acid from mannitol, but there is no action on dulcitol or sorbitol. The *pentoses* arabinose and xylose are slowly attacked.

Evidence of the power to hydrolyse *fats* is seen in acid production from tributyrin.

Oxidation and Reduction.

In pneumococcal cultures, in sterile filtrates, and also under certain conditions, in suspensions of living pneumococci and in sterile autolysates, active processes of oxidation or reduction occur, the direction of the reaction depending on the tension of atmospheric oxygen in the fluid in question.

The conversion of hæmoglobin to methæmoglobin is an example of the oxidation process, since methæmoglobin is an isomer of oxyhæmoglobin in which the oxygen is more firmly bound, the iron in the molecule being converted to the ferric state. As we have seen in the case of living cultures, this change only occurs under aerobic conditions. It also occurs in sterile culture filtrates (Morgan and Neill, 1924) and in sterile autolysates, provided these have not been prepared from washed pneumococci. The first stage in the reaction is a dissociation of the oxyhæmoglobin. The reduced hæmoglobin is then oxidized to methæmoglobin and at low oxygen tensions this process is reversed, hæmoglobin again being formed (Neill and Avery, 1924[1]; Neill, 1925; Neill and Hastings, 1925). Similarly at low oxygen tensions methylene blue is reduced to its colourless form. The exact mechanism of these reactions is not yet fully understood, but it is probable that the oxidation and reduction depend on the same factors. When reduction occurs the source of oxygen or the hydrogen acceptor is the substance undergoing reduction (Avery and Neill, 1924[3]).

McLeod and Gordon (1922) have shown that hydrogen peroxide is formed in pneumococcal cultures under conditions of good aeration, and that as no catalase is produced by the pneumococcus the peroxide may accumulate to such a degree of concentration as to cause the death of the micro-organism. The favouring effect of red blood corpuscles and of fresh vegetable tissues (Morgan and Avery, 1923) in accelerating and prolonging the active growth and delaying autolysis is no doubt in part due to their rich catalase content. It is suggested by Platt (1927[1]) that the favouring effect of gelatin may be due to the decreased solubility and rate of diffusion of oxygen to which it gives rise.

Hydrogen peroxide does not appear to an appreciable extent in cultures until after the period of rapid growth (Morgan, 1924; Platt, 1927[2]), and it continues to increase in conditions which are incompatible with growth (Avery and Neill, 1924[1]). Its appearance is dependent on the presence of certain thermostable substances in meat extract. Platt (1927[2]) has shown that it is not formed to an appreciable extent in well-aerated cultures in gelatin peptone-water. He finds that glucose and sodium lactate can to some extent replace meat extract in this respect. Similarly, oxidation processes do not take place in autolysates prepared from washed pneumococci; the system requires to be completed by the addition of some of the culture fluid or of yeast extract (Avery and Neill, 1924[2]). Living washed pneumococci are activated by glucose, but sterile autolysates are not (Neill, 1925). Avery and Neill (1924[2&3]) and Neill and Avery (1924) suppose that certain easily oxidizable constituents of unknown nature in

meat extract are oxidized to organic peroxides. Platt (1927[2]) criticizes this view and maintains that the thermostable activating substances contained in meat extract act as hydrogen donators to molecular oxygen with the formation of hydrogen peroxide, and that they are of the nature of lactic acid or similar substances. The intracellular substance of pneumococcus which determines the oxidation-reduction process is thermolabile and is inactivated by exposure to air, being itself apparently subject to oxidation (Neill and Avery, 1925).

Intracellular Substances.

Proteins. When dilute acetic acid is added to a clear solution prepared from washed pneumococci a precipitate forms which is soluble in excess of acetic acid, and is evidently of the nature of *nucleoprotein.* After filtration and partial neutralization the filtrate again gives a slight precipitate on boiling, indicating the presence of *albumin* or *globulin.* The nucleoprotein forms the bulk of the bacterial cytoplasm and appears to be distinct from the capsular substance. As shown by precipitin reactions it is serologically identical in all pneumococci, including the degraded 'R' forms which have lost their capsular substance (see p. 211). It appears in solution in cultures only after they have begun to autolyse (Avery and Morgan, 1925; Reimann, 1926).

Type-specific polysaccharides. Dochez and Avery (1917) showed that at an early stage in the growth of pneumococci a substance appears in the culture fluid which gives precipitin reactions with the homologous immune serum. They found this substance also in the urine of pneumonia cases. This specific substance can be boiled without destroying its reactive property; it is precipitable in acetone, alcohol and ether, and the precipitate is readily soluble in water. It does not diffuse through parchment, and its serological activity is not affected by digestion with trypsin. Working with very large quantities of broth cultures, Heidelberger and Avery have obtained this specific substance in sufficiently large amount and sufficiently pure for chemical analysis, and have shown that the substance corresponding to each serological type is a polysaccharide of different constitution for the three types. Although they do not claim that their various preparations obtained in different ways are pure and individual chemical substances, they have shown that these preparations are very constant in their physical and chemical properties; in particular, it has not been possible to separate the polysaccharide from the active specific substance.

These polysaccharides differ from those of the starch glycogen group in giving no reaction with iodine and in their resistance to the ordinary carbohydrate-splitting enzymes. They are hydrolysed only by boiling with strong acid.

The polysaccharide of Type II is dextrorotatory (+55°), is built up of glucose units and is a weak acid (Heidelberger and Avery, 1923, 1924); that of Type III is lævorotatory (−33°), is the salt of a rather strong

insoluble acid, and is built up of units of a di-saccharide acid which contains one carboxylic group and one aldehyde-reducing group in the molecule (Heidelberger and Goebel, 1926, 1927). The polysaccharide of Type I differs sharply from the other two in containing nitrogen and in possessing basic as well as acid properties. It is dextrorotatory (+300°), and gives none of the usual protein colour-tests. Treatment with nitrous acid liberates half the nitrogen, and with that the property of reacting with Type I serum vanishes (Heidelberger, Goebel and Avery, 1925). There is reason to believe that the specific polysaccharides are associated with the capsular substance. They are not present in the degraded ' R ' forms. Their antigenic relationships are considered on p. 205.

Purpura-producing substance. Another heat-stable body occurs in pneumococcus culture fluids, the presence of which is detected by the production of purpuric hæmorrhages in the ears, feet and tail of mice injected with the killed culture or with filtrates. The substance is not found in young cultures before autolysis has begun ; it occurs in larger amounts in autolysed suspensions of washed pneumococci, and is to be regarded as a constituent of the pneumococcal cell. It is not precipitated from the pneumococcal extract with the nucleoprotein on the addition of acetic acid, or on boiling, and it appears to be of the nature of a proteose. It occurs in ' R ' forms, and is, therefore, associated with the cell-body, and not with the capsule. The susceptibility of individual mice varies in a marked degree, and is capable of increase by selective breeding, since mice which react recover without permanent damage (Julianelle and Reimann, 1926, 1927 ; Mair, 1928).

Hæmolysin. The pneumococcal cell contains a hæmolysin for the red blood corpuscles of various animals. The occurrence of hæmolysis on blood-agar plates, best seen when they are incubated under anaerobic conditions, has already been referred to. Since corpuscles in which the hæmoglobin has been oxidized to methæmoglobin are no longer sensitive to the hæmolysin its action is obscured in aerobic cultures. In sterile autolysates of washed pneumococci, in which oxidation does not occur, its action can be easily demonstrated. It is apparently protein in nature, possesses the power of inducing antibody formation, and is destroyed by tryptic digestion (Cole, 1914[1]). It is destroyed by heating at 55° C. for 10 minutes, and when exposed to air in extracts made from unwashed pneumococci it is oxidized to an inactive form (Avery and Neill, 1924[4]). On re-establishing anaerobic conditions it again becomes active (Neill, 1926).

Intracellular enzymes. Avery and Cullen (1920[1]) have shown that sterile extracts of pneumococcus contain an enzyme which hydrolyses with striking activity proteoses and peptones. It attacks to some extent the intact proteins casein and fibrin, but is without demonstrable effect on albumin and gelatin. The optimum H ion concentration for its action lies between pH 7 and pH 7·8 (which also represents the optimum reaction for the growth of pneumococcus), and it thus belongs to the same class of

ferments as *erepsin*. On heating there is a progressive loss of activity as the temperature is raised, and its action is completely abolished in 10 minutes at 100° C. The same authors (1920[2]) report the presence in cell extracts of a *lipase* of marked activity as shown by the acid liberated by its action on tributyrin. The optimum H ion concentration for the action of this enzyme is about pH 7·8, and it is completely inactivated by heating for 10 minutes at 70° C.

Avery and Cullen (1920[3]) also describe enzymes in the cell extract which hydrolyse saccharose, starch and inulin (*invertase, amylase*). These are active within the limits pH 5 to pH 8 with an optimum at pH 7. They are destroyed by heating for 10 minutes at 55° C. No enzyme could be demonstrated capable of fermenting glucose with acid formation, although this action is characteristic of the living cell. Neill and Avery (1924[2]) report the presence of *raffinase*, and Fleming and Neill (1927) that of *maltase* and *lactase* in the pneumococcal cell extract. The presence in the pneumococcal cell of an *autolysin* (p. 169), and the absence of *catalase* (p. 172) have already been mentioned.

Neill and Avery (1924[2]) have shown that certain of the pneumococcal enzymes are destroyed when freely exposed to the action of air in extracts in which the oxidation-reduction system is complete. They are not themselves reactive with molecular oxygen, as is shown by their stability in extracts made from washed pneumococci. The saccharolytic enzymes (invertase, raffinase, inulase and amylase) are the most easily inactivated by aeration of the unwashed extracts, but the bacteriolytic enzyme (autolysin) is also reduced in activity. Similar treatment is without effect on the active concentration of the lipase and peptonase. There is thus a certain parallelism between the resistance of these enzymes to oxidation and to heat.

Neill and Fleming (1927) have further shown that the bacteriolytic activity of oxidized pneumococcus extracts can be restored by establishing anaerobic conditions by the addition of living anaerobic bacilli.

Discovery of Micro-organisms in Pneumonia.

By W. Bulloch.

The occurrence of micro-organisms in pneumonia was noted by Klebs (1875) and Eberth (1881). In the latter year R. Koch published photographs of diplococci in sections of the lung and kidney of a case of pneumonia complicating relapsing fever. Leyden (1882) found similar cocci during life in blood obtained from pulmonic puncture in a case of pneumonia. Friedländer (1882) again found cocci in pneumonic exudate in eight cases. The modern ideas on the bacteriology of pneumonia date, however, from the publication of his paper in 1883. In this work he refers to the examination of more than 50 cases of pneumonia, in the majority of which he found the microscopic evidence of cocci. In one case he successfully isolated the organism in culture, and described its characters. He was

of opinion that the coccus was distinguishable from other cocci in three ways : (1) by its possession of a capsule, (2) by the characteristic appearance of the cultivation in gelatin, and (3) by its pathogenic properties, of which its failure to produce disease in rabbits was particularly emphasized. Friedländer described the organism now called after him with considerable accuracy. In a series of 25 cases of pneumonia, L. Talamon (1883), in the Hôtel-Dieu, Paris, found repeatedly an elongated ellipsoidal coccus the shape of which he compared to a lancet of the kind known as ' à grain d'orge ' or to the flame of candle. He grew it in meat extract fluid, and found that the cultures were pathogenic for 16 out of 20 rabbits. He clearly expressed the view that pneumonia is an infective disease caused by a special microbe. The infective character of pneumonia was also strongly emphasized by Jürgensen (1884). It was in this year that Albert Fraenkel's classical works on the pneumonia cocci began to appear. He found that they agreed with the description given by Talamon rather than with that of Friedländer. He obtained cultures quite unlike the ' nail ' cultures described as characteristic by Friedländer (1883), and he found that the new coccus was highly pathogenic for rabbits. Fraenkel's chief papers appeared in 1886, and contained an exhaustive account of the ' Pneumoniecoccus '. He showed that it agreed with the coccus which Pasteur, Chamberland and Roux (1881[2]) and Sternberg (1881) had found in the blood of rabbits inoculated with human saliva. This microbe of sputum septicæmia was an elongated coccus surrounded by a capsule. Fraenkel showed that it required a relatively high temperature for its growth and was very pathogenic for rabbits. Fine dewdrop-like colonies appeared when it was grown on blood-serum, and the virulence was rapidly lost in artificial media. An identical organism was grown from five cases of pneumonia, and even from empyema. He also showed that the coccus is Gram-positive, whereas the Friedländer organism was Gram-negative. Confirmation of Friedländer's observations was made by Mendelsohn (1884), Emmerich (1884), Foà and Rattone (1885) and Senger (1886), but a complete confirmation of the importance of the Talamon-Fraenkel coccus was made especially by Weichselbaum (1886) in an exhaustive analysis of the bacteriology of no fewer than 129 cases of pneumonia of different types. By far the commonest organism in pneumonia was found to be the pneumococcus of Fraenkel. Weichselbaum, however, showed that at least three other organisms may cause pneumonia. The work of Talamon, Fraenkel and Weichselbaum laid the basis of all our subsequent knowledge of the important microbe commonly called pneumococcus. Further additions were made by Netter (1890) and Kruse and Pansini (1892), and since then the literature of every aspect of the pneumococcus problem has grown to enormous dimensions.

Pathogenic Action.

By J. W. McLeod.

Natural Infection in Animals.

It would appear from the fact that there is no mention of the pneumococcus in a modern text-book of veterinary bacteriology, such as that of Bongert (1922), that lesions in animals due to this organism are neither very frequent nor very important. There are, however, some scattered references in the literature to spontaneous pneumococcal infection amongst the types of animal commonly used in the laboratory for experimental purposes. Thus, Blake and Cecil (1920[3]) found that monkeys in captivity and overcrowded developed spontaneously a pneumonia indistinguishable from that which occurs in man. It was usually caused by a Group IV pneumococcus.

Several references to spontaneous lesions in animals are also found in the French literature. Grenier (1912) quotes extensively from Nicolle's work, in which it was a common experience to find a generalized or local pneumococcus infection arise as a complication when various toxic agents or bacterial cultures were injected to guinea-pigs. In studying experimental glanders it was observed that an inflammatory condition of the nose which occurs in this animal was due to a pneumococcus. Grenier noted similar ' émergences ' of pneumococci in rabbits and once in a horse. Cotoni, Truche and Raphael (1922) obtained results of the same nature. Th. Smith (1913) ascribes a like role to the pneumococcus in spontaneous disease in the guinea-pig. He found the *B. bronchisepticus* of McGowan the commonest cause of spontaneous pneumonia in that animal, but the pneumococcus frequent as a secondary invader, following the former and sometimes present in pure culture.

Bruckner and Galasesco (1916) describe a spontaneous laboratory epidemic amongst their guinea-pigs which occurred in two successive years and killed off 160 animals in one month in the first year. Lack of appetite, emaciation and sluggish movements were the chief symptoms, and an encapsulated pneumococcus was recovered from the tissues after death.

Petrie (unpublished) has observed numerous spontaneous pneumococcal infections during the last four years amongst the breeding stock of guinea-pigs at Elstree. In these infections the young were still-born, and the sows succumbed subsequently to a sort of puerperal infection sometimes complicated by pneumonic pulmonary lesions. The pulmonary lesions also appeared occasionally in the boars. The strains isolated from these animals were found by Mair and Griffith to fall in Group IV, and in this respect Petrie's findings correspond to others recorded on the same subject by Meyer (1928).

Holman (1916), however, examining bodies of animals which died spontaneously in a large stock of guinea-pigs kept under good conditions

remote from hospitals, found the pneumococcus very rarely—three doubtful strains in all. He observed spontaneous pneumonia in the animals, but found it to be a streptococcal lesion.

Experimental Infection in Animals.

The lesions produced by pneumococci which gain access to the animal body by injection, by inhalation or in other ways are chiefly determined by the susceptibility of the animal on the one hand and the virulence of the particular strain on the other. The site of injection does affect the result, but not appreciably in the case of highly virulent strains acting on susceptible animals. Cafeiro (1914) gives the following lethal doses for different animals. Rabbit, 0·0000001 c.cm. subcutaneous ; guinea-pig, 1 to 2 c.cm. subcutaneous and 0·1 to 2 c.cm. intraperitoneal ; dog, 8 c.cm. subcutaneous, 2 c.cm. intravenous, but 8 c.cm. intraperitoneal not fatal ; pigeon, entirely refractory. The extreme susceptibility of the mouse to the pneumococcus is a commonplace of laboratory experience and forms the basis for testing the efficacy of antisera in the methods of Neufeld and Händel (1912) and of Avery, Chickering, Cole and Dochez (1917).

Although the mouse may be killed by a smaller dose than the rabbit, it is an open question which animal is the more susceptible in view of their great difference in weight, as Cotoni, Truche and Raphael (1922) point out. The last named take pathogenicity for the guinea-pig as a criterion of high virulence in selecting a strain suitable for immunization. Bull's (1916) figures showing the relative lethal dose of a virulent strain of pneumococci for dogs and rabbits respectively bear out those of Cafeiro, and the relatively refractory state of dogs to this infection is remarked by Idzumi (1920) and other observers. The peculiar resistance of the pigeon appears to be related to its normally high body temperature, since Findlay (1922) was able to repeat Strouse's result that lowering the bird's temperature by injection of pyramidon rendered it susceptible to large intraperitoneal injections of pneumococci, and found in addition that a less drastic method of lowering the temperature, i.e. three weeks on a diet free from vitamin 'B' also rendered it susceptible to pneumococcal infection.

The varying effect of an injection of pneumococci as it was determined by the virulence of the strain and the resistance of the animal was carefully described by Eyre (1901), his injections were made subcutaneously to the rabbit. With high virulence and low resistance death occurred within 48 hours from general septicæmia ; the local lesion was slight, usually œdema and more rarely hæmorrhage. With a strain of lower virulence or a rabbit partly immunized death did not occur earlier than the fourth to seventh day, and the local lesion was pronounced, consisting of marked infiltration with fibrin, leucocytes and blood, and, more remotely, serous exudate. When a highly immunized animal was injected with a virulent strain or a susceptible animal with an avirulent strain the

result was trivial or entirely local—development of an abscess which pointed. The chief interest in experimental inoculation of animals, however, has centred on the numerous attempts to reproduce pulmonary lesions resembling lobar pneumonia as it occurs in man. Such lesions rarely if ever occur as a result of subcutaneous, intraperitoneal or intravenous inoculations. Thus Kiralyfi (1910) injecting guinea-pigs subcutaneously found no more definite pulmonary lesions than patchy œdema and infiltration with round cells of the interstitial tissues, with, in some cases, accumulation of blood and exudate but no fibrin in groups of alveoli.

Many attempts have been made to produce pneumonia by direct inoculation to the respiratory tract. The methods which have been employed for this purpose with varying success have been (1) introduction of culture to the larger bronchi by means of a tracheal catheter, (2) direct introduction of culture to the trachea by puncture with needle and syringe, (3) exposure of the animals to a vapourized culture of pneumococcus in a confined space, (4) direct injection to the lung through the chest wall. Lamar and Meltzer (1912) and Wollstein and Meltzer (1912, 1913) in an extensive series of investigations on the effect of introduction of large amounts of fluid culture to the trachea of dogs, and insufflation of the fluid so that it should reach at all events the finer bronchi found that a frank lobar pneumonia could be produced in this way. They showed, moreover, that it was a lesion peculiar to the pneumococcus, since it was not produced either by injections of cultures of streptococci or of *B. influenzæ*, nor yet by injections of large quantities of culture of a saprophytic bacterium such as *B. megatherium*. Gay and Rhodes (1921) adopting similar methods produced red and grey hepatization in the lungs of rabbits although these died within three days of septicæmia. The lesion in this case also was distinct from that produced by streptococci. Armstrong and Gaskell (1914, 1921) compared the results of various methods of inoculation in the rabbit and got conditions resembling lobar pneumonia, even when the animal died within 24 hours, if a virulent culture was insufflated per catheter introduced deeply into the trachea, but they could not produce such lesions by (*a*) injection per needle to trachea, (*b*) direct injection to lung, (*c*) by intravenous injection. Gaskell (1925) and (1928) extended and confirmed these results.

The observations of Blake and Cecil (1920[1 & 2]) on experimental pneumonia were carried out on the monkey by the second method—tracheal puncture. Amongst 31 monkeys inoculated in this way with varying amounts of Type I culture, 26 developed pneumonia and 21 died, whereas of 6 injected with other types, 4 developed pneumonia but none died. They were never able to produce pneumonia, however, by intravenous injection or by infection of the nasal passages. Histological examination of animals killed at short intervals after injection suggested that pneumococci reached the lung by the peribronchial connective tissue and lymphatic system.

The third method of infection, that by inhalation, has been investigated by Griffith (1926), in this country. He exposed mice to a nebulizer spray of virulent culture, and found that he got a high percentage of fatal septicæmias but very little consolidation of the lung. The most extensive work on this line, however, is that of Stillman (1923, 1924[1 & 2]), Stillman and Branch (1924, 1925), Branch and Stillman (1924, 1925) with mice, and of Stillman and Branch (1926) with rabbits. In these experiments, 10 to 15 c.cm. of fluid culture were sprayed into a box with an atomizer, and the mice or rabbits were then confined in this atmosphere for one hour. Large numbers of mice and rabbits were investigated and the following points were determined: (1) the pneumococci reached the peripheral parts of the lungs within two hours, (2) the mice were resistant, only about 2 per cent. dying in a large series, and these had developed general septicæmia, (3) the rabbits were less resistant as 38 per cent. died from septicæmia. The insusceptibility of the mice could be overcome, however, if they were intoxicated with an intraperitoneal injection of alcohol. Amongst mice exposed to the culture spray while intoxicated 20 to 50 per cent. died of pneumococcal infection. It was only, however, in 20 to 50 per cent. of mice which had developed some degree of immunity as the result of earlier exposures to pneumococcus spray that lesions suggestive of lobar pneumonia were observed. Red hepatization was not met as a rule unless the mouse survived till the second day after exposure, and grey hepatization rarely appeared except in mice surviving till the third day. Amongst 41 intoxicated mice which died after one exposure, no more definite pulmonary lesions than congestion or more rarely interstitial inflammation and sero-fibrinous pleurisy were observed.

In observations (unpublished) made by the writer and Gordon, it has been found much more difficult to produce pneumonia in the monkey by introduction of pneumococcal cultures to the trachea or bronchi than might be supposed from reading the accounts of the work quoted above. In these experiments—nine in all—the cultures used included (*a*) pneumococci recently isolated from empyemata in man, (*b*) one of these strains after passage through a monkey which was killed by a large intraperitoneal dose, and (*c*) a Type I strain of high virulence for mice obtained from Dr. F. Griffith. The earlier experiments were made by intratracheal injection with needle and syringe, the latter by insufflation of 1 c.cm. of culture by rubber catheter passed to the main bronchi. That the Type I strain, at all events, was sufficiently virulent was proved by the death of one of the monkeys within three days with pneumococci present throughout the body and an acute peritonitis. In no instance, however, was any lesion produced which remotely resembled lobar pneumonia. Most of the animals developed a temperature of 1 to 2° C. associated with a distinct leucocytosis, both of which persisted for 1 to 4 days. The monkeys which reacted in this way were killed within 24 hours of defervescence and portions of both lungs examined histologically. Nothing more than interstitial infiltration with catarrhal exudate in some of the alveoli was

ever noted. The cause of this discrepancy with the experimental work published by Blake and Cecil is obscure. It may be that their results depended on peculiarities of the pneumococcal strains used, or of the type of monkey injected. It is obvious, however, that the very important demonstration carried out by Cecil and Blake (1920[3]) on a small series of animals and controls of the curative effect of antiserums on pneumonia due to Type I pneumococci may be difficult to repeat, although these results have been confirmed and extended by Cecil and Steffen (1925[1 & 2]) who describe cures of pneumonia in the monkey due to both Types I and II strains, and obtained with Huntoon's polyvalent pneumococcus antibody solution. In this connection it is noteworthy that Christie, Ehrich and Binger (1928) working in the Rockefeller Hospital, refer to the recognized difficulties in producing experimental lobar pneumonia.

Intrathecal injections. Idzumi (1920) observed that meningitis was not produced in rabbits or dogs after intravenous inoculation of pneumococci of Types I, II or III, but that the rabbits might develop it if a simultaneous lumbar puncture was performed. In dogs, however, meningitis was only produced by intrathecal injection, and even then was obtained less regularly and was less marked than in rabbits treated in the same way. In Bull's (1916) experience, however, a number of dogs in which he produced pneumococcal septicæmia by large injections of virulent pneumococci developed meningitis. Some of these recovered and others died. Apart from the development of meningitis the dogs almost always recovered. Stewart's more recent work on this subject is described on p. 231.

Introduction to lower alimentary tract. McCartney and Fraser (1922) failed in all attempts to produce peritonitis by introducing large amounts of virulent culture to rabbit stomachs by tube, even when the gastric secretions were neutralized with $NaHCO_3$ and peristalsis inhibited with opium, and Eguchi (1925) only produced pneumococcal septicæmia in 1 of 15 mice to which culture of Type I was fed.

Intracorneal inoculation. Chaillous and Cotoni (1925) found that this method of inoculation was followed in some cases by development of a grey area of necrosis, which was absorbed, in others a progressive lesion with accumulation of inflammatory exudate in the anterior chambers and ultimate destruction of the eye resulted, provided that the animal did not die early of a generalized septicæmia.

Cutaneous inoculation. Panton and Benians (1925) found that the usual result of rubbing virulent pneumococcus culture into the lightly scarified skin of the rabbit was the development of an erythematous area suggestive of erysipelas, but no lesion of this kind was obtained in mice treated similarly.

Natural Infection in Man.

Although the pneumococcus may be responsible for a variety of lesions in the human body, it is as the common and almost unique cause of acute

lobar pneumonia that it has become the focus of an intensive investigation that has few parallels in bacteriological literature. In the Rockefeller Monograph on the subject Avery and Chickering, Cole and Dochez (1917) state that amongst 480 cases of lobar pneumonia in which the bacteriological ætiology was clearly determined, 454 were due to the *Diplococcus pneumoniæ*. The remaining 26 were caused by streptococcus, staphylococcus, *B. influenzæ* and Friedländer's bacillus or by mixed infections with two or more of these.

The increasing importance of pneumonia as a cause of death is emphasized by Lord (1922) and by Glynn and Digby (1923) since the mortality for which it is responsible now exceeds that of phthisis. Kelly (1926) has presented extensive statistical data showing *inter alia* the much higher incidence of pneumonia amongst men.

The Respiratory Tract as a Normal Habitat.

Two obvious questions present themselves in connection with pneumococcal infections of the respiratory tract : (1) Is the pneumococcus found in the normal trachea, bronchi or lungs? (2) Does it form part of the numerous and varied bacterial flora of the normal mouth ?

It is altogether probable on *a priori* grounds that pneumococci reach the smaller bronchi from time to time, especially in mouth breathers. This opinion is strengthened by the experiments of Stillman and Branch (1926) already referred to. The claim which has sometimes been advanced (Dürck, 1904), that bacteria are constantly present even in the lung alveoli, cannot be as readily accepted. Norris and Pappenheimer (1905) showed that *B. prodigiosus* introduced into the mouth of dead patients could be detected in the periphery of the lung in more than 50 per cent. at autopsy and drew the obvious conclusion that post-mortem bacteriological findings in the lung were unreliable as an indication of the bacteria present before death. Luetscher (1915) also emphasizes the caution necessary in accepting post-mortem cultures as evidence of infection of the lung during life. He maintains that the pneumococcus invades the lung very rapidly after death since it is found as frequently in normal as in pneumonic lungs at autopsy. Ritchie's (1901) findings with regard to the bacterial content of the bronchi of children dying from bronchitis in Vienna do not support Luetscher's however. Ritchie claimed that pneumococcus and *B. influenzæ* had ætiological significance precisely because they were found in bronchitis and not in the absence of bronchial lesions, whereas streptococci and coliform bacilli appeared as frequently in the absence of a bronchial lesion as when one was present. There is little doubt, however, that Luetscher is right when he says that the weight of experimental evidence goes to show that the normal lungs and bronchi are usually sterile.

There appears to be complete unanimity in answering the second question in the affirmative : good evidence for the presence of the pneumococcus in the normal mouth has existed for nearly 50 years, and

all the more recent investigations in which accurate methods of identifying the pneumococcus have been used have established the conclusion that this organism is not infrequently present in normal mouths and throats. Whether it is to be considered a constant inhabitant of the normal upper respiratory tract or merely a frequent invader is a more difficult question to decide. Meyer, Pilot and Pearlman (1921) in investigating the throats of 49 children who had had tonsillectomy performed and 68 who had not had this operation, found the pneumococcus present in 32 per cent. in both series. Williams, Nevin and Gurley (1921) found pneumococci in the nasopharynx of 20 per cent. of normal individuals. Lyall (1915) notes the presence of pneumococci in 40 per cent. of tubercular sputa. Gordon (1921) examining 46 normals found the pneumococcus in 21 per cent. Pilot and Pearlman (1921) investigating the bacteriology of excised tonsils and adenoids, found pneumococci present in 60 to 70 per cent.; but Howarth and Gloyne (1923) only found pneumococci in 18·7 per cent. of unhealthy tonsils excised. Bloomfield (1921) who made a weekly bacteriological examination of a group of 11 people over a period of at least 5 weeks, only discovered the pneumococcus in 3, and in those only transiently; and Stillman (1922) contrasts the presence of the pneumococcus in the noses of 40 per cent. of patients with pneumonia, and its relative rarity in normal people or in those suffering from coryza.

The general impression gathered from these records is that the pneumococcus is present in the throats of at least 20 per cent. of normal individuals. This finding may be explained in one or two ways: either the pneumococcus is one of the constant inhabitants of the throat and escapes observation in a considerable percentage of individuals because it is present in very small numbers, or it is merely a frequent invader. The observation of Pilot and Pearlman (1921), that pneumococci may be present in pure culture or in large numbers in the excised adenoids when nasopharyngeal swabs from the same individuals had yielded cultures in which they were scanty suggests that the first view is correct.

Role of the Pneumococcus in Minor Inflammatory Conditions of the Upper Respiratory Tract.

It is difficult to believe that, if the pneumococcus were the cause of 'the common cold', i.e. of the most frequent catarrh of the upper respiratory tract, overwhelming evidence would not already have been brought forward to prove its connection with this complaint. Against such a supposition are the finding of Williams, Nevin and Gurley (1921) that hæmolytic streptococci and *B. influenzæ* are, equally with the pneumococcus, more frequently present in the throats of sufferers from 'cold' than in those of 'normals', and also those of Blake and Cecil (1920[1]) who never produced coryza by inoculating highly virulent pneumococci on the nasal mucous membrane of monkeys, although the bacteria persisted there for considerable periods of time. It is not improbable, however, that the terms 'common cold' and influenza cover

a group of infections of the upper respiratory tract, and that amongst these pneumococcal infections hold an important place. In Hiss and Zinsser's text-book of Bacteriology (1922) it is stated, that in man the most frequent lesion produced by the pneumococcus is acute lobar pneumonia. This, in the writer's opinion, is incorrect, and to him it seems that the most frequent lesion produced by the pneumococcus is a catarrhal condition of the upper respiratory tract involving the larger bronchi, associated with slight or considerable pyrexia, and often popularly described as 'influenza'.

Most theories of the genesis of pneumonia suppose a penetration of the mucous membrane of the upper respiratory tract followed either by direct extension to the lung through the tissue around the hilum, or by a preliminary septicæmia with localization in the lung subsequently. When an infection of that type is aborted owing to insufficient virulence of the invading micro-organism a temporary invasion of the mucous membrane and considerable reaction, involving fever, on the part of the person attacked must occur. Certainly in the attempts of the writer and Gordon to produce lobar pneumonia in monkeys, already referred to, and in similar experiments in dogs a condition corresponding to certain clinical 'influenzas' was the only one frequently produced, while a minority of animals failed to react at all or developed acute septicæmia.

The evidence in the literature for the existence of minor inflammations of the upper respiratory tract due to the pneumococcus is considerable, and without attempting to quote the whole of it a number of examples may be given. Beck and Stokes (1907) record 56 cases of epidemic catarrh—temperatures 99·5 to 102° F.—and involvement of conjunctiva, nose, pharynx, nasopharynx and larynx, but none of bronchi or lungs; in some of these pneumococci were present in pure culture and in almost all in mixed culture. Some of the pneumococci were tested on rabbits and mice and found pathogenic, and although the bacteriological examination was, from the standpoint of to-day, incomplete, there is little doubt that they were dealing with the pneumococcus. Walter (1910) found the pneumococcus abundant in 7 per cent. of the cases of rhinitis which he examined, and these were the most severe cases of his series, being associated with fever and being the class of case usually described as 'influenza'. Walb (1913) points out that the seasonal catarrh seen for the previous 10 years in Bonn, and clinically described as 'influenza', but differing from true influenza in gradual onset, had not been found to be associated with the influenza bacillus, and was actually a pneumococcus infection; he does not, however, give details of his bacteriological investigations.

Lenz (1917) observing an epidemic of pneumonia in a prisoners' camp in Bavaria, which reached its height in warm spring weather in 1915, could only associate it with a simultaneous and very widespread epidemic of febrile catarrh, since in subsequent years, and with much worse weather conditions, very few cases of pneumonia occurred. Valentine (1918)

found the pneumococcus in 44 of 65 colds examined ; 2 of these gave cultures in which 75 per cent. of the colonies were Type I pneumococci and were unusually severe infections with temperature of 102° F. More elaborate observations on this subject, however, have been made by Gordon (1921). He found pneumococcus present in the upper respiratory tract in 21 per cent. of ' normals ', in 36 per cent. of cases of ' cold ', and in 47 per cent. of cases of bronchitis. Moreover, he found that the strains from cases of rhinitis exceeded in their virulence to animals those obtained from ' normals '. Lastly he describes a localized epidemic of sore throats with unusual clinical features occurring amongst school-children, and associated with Group IV pneumococci, which were found to be of similar immunological type in all cases, although distinct from the pneumococci of the same group isolated from healthy children in the school. In these observations there is at all events sufficient evidence for supposing that extended application of more careful bacteriological methods would enable us to differentiate a considerable group of febrile catarrhs of the upper respiratory tract as pneumococcal infections.

Pneumonia and Broncho-pneumonia.

Genesis of pneumonia.

There are three obvious ways in which a lobar pneumonia might be developed. (1) An invasion of the lung entirely through the respiratory passages either by a continuous downward extension of an inflammatory catarrh starting in the larynx, or, where fine moist particles loaded with virulent pneumococci had been inhaled, originating in the secondary bronchi and extending outwards from there. (2) A penetration of the mucous membrane of the trachea or bronchi, with subsequent spread through the connective tissue framework of the lung and its lymphatic system. (3) Penetration of any part of the upper respiratory or even of the alimentary tract with development of septicæmia and localization in the lung.

There is so much evidence that the outstanding feature of the pneumococcus is its invasive power that it is difficult to suppose it spreading through the lung along the surface of the air passages to their finest ramifications, and to see how such a method of spread could result in anything but a lesion of irregular and lobular distribution. To discriminate between the second and third possibilities is less easy. That some degree of septicæmia is a frequent feature of pneumonia is generally accepted : Baermann (1914) obtained 30 to 64 per cent. of positive blood-cultures according to the technique used ; Avery, Chickering, Cole and Dochez (1917) got 30 per cent. in a very large series ; Clough (1917) got 33 per cent. ; Lyon (1922) got 14 per cent. in lobar pneumonia in children ; and Cotoni, Truche and Raphael (1922), 20 to 30 per cent. Although it may be possible to demonstrate the presence of pneumococcus in the blood in some cases before a pulmonary lesion is clinically obvious, it does not follow that septicæmia is a necessary

preliminary to pneumonia. It may only be an overflow phenomenon from the pulmonary lesion and a simultaneous development.

Armstrong and Gaskell (1921) and Gaskell (1925) maintain that the pulmonary lesion which develops as a consequence of a pneumococcal septicæmia is quite distinct from lobar pneumonia. Baermann (1914), whose work on blood culture was very extensive, records one or two cases where he had opportunity of making blood cultures repeatedly from the onset of illness up till the first clinical evidence of consolidation, and yet got no positive results, and Schottmüller (1905) records two cases in which blood culture was negative on the first day of disease. These observations are good evidence that septicæmia is not a necessary precursor of the development of the disease. Further evidence which points in the same direction is the low percentage of positive blood cultures in mild cases. The universal experience that experimental pneumonia cannot be produced by intravenous inoculations of the pneumococcus strengthens this conclusion.

The careful experimental work of Blake and Cecil (1920[1 & 2]), in which monkeys were killed at various stages in the development of pneumonia consequent on intratrachael inoculation and their tissues submitted to histological examination, indicates a direct extension through the tissues about the hilum outwards to the lung, although a simultaneous septicæmia was invariable. The most probable explanation of the development of the pneumonic lesion would therefore appear to be that virulent pneumococci gaining access to the trachea or bronchi penetrate some part of the mucous membrane more deeply and then invade the peribronchial tissues and by continuous extension all the adjacent lung tissues, until their progress is arrested by an interlobar septum or by the concentration of the body's antibacterial agencies; some degree of simultaneous septicæmia being the rule, but not invariable. It is quite in keeping with such a scheme that peculiarly sensitive individuals like some of the French African troops should develop a grave septicæmia so rapidly that the pulmonary lesion is rarely fully developed (Cotoni, Truche and Raphael, 1922).

Genesis of broncho-pneumonia and effects of association with other bacteria.

The first question that arises with regard to broncho-pneumonia is whether it is an uncomplicated pneumococcal lesion. There is very little evidence that broncho-pneumonia can be produced experimentally with the pneumococcus, the lesions observed in mice by Branch and Stillman (1924) were interstitial pneumonias except in the rarer instances in which red or grey hepatization was produced, and Wollstein and Meltzer (1912, 1913[1, 2 & 3]) in their work on dogs particularly emphasize the point that it was a lobar pneumonia which was produced whatever the grade of virulence of the pneumococci, whereas streptococci and *B. influenzæ* insufflated in the same way caused broncho-pneumonias. Armstrong and Gaskell (1921) report one instance in which injection of

5 c.cm. of culture of pneumococcus of moderate virulence to the bronchi of a rabbit produced a condition resembling broncho-pneumonia. This method of infection, however, does not correspond to anything that is likely to occur frequently in the development of disease in man. In later work, however, Gaskell (1925, 1928) has shown that insufflation to the main bronchi of rabbits of considerable, although smaller, quantities (1 c.cm.) of pneumococcal cultures of a certain grade of virulence will produce limited broncho-pneumonias about the centre of the lung.

The outstanding features of the occurrence of broncho-pneumonia in man are (1) its association with influenza epidemics, (2) its frequency amongst small children. Rogers (1925) reviewing his extensive experience of post-mortem work in India states that apart from periods of epidemic influenza broncho-pneumonia is rarely met in adults, although lobar pneumonia is common. This is also the experience in Europe. Exception is made of some localized epidemics of bronchiolitis and broncho-pneumonia in the period immediately preceding the recent pandemic of influenza (Hammond, Rolland and Shore, 1917; Abrahams, Hallows, Eyre and French, 1917; McLeod, Ritchie and Dotteridge, 1921); in these the role of the pneumococcus was obviously secondary, or accessory, to that of the influenza bacillus. In view of such findings accounts such as that of Hirsch and McKinney (1919) of epidemics of pneumococcal broncho-pneumonia, which occurred during the period of the influenza pandemic and in which the media shown by recent experience to be best for isolation of *B. influenzæ* were not used, must be accepted with reserve. There is a good deal of evidence to show that the pneumococcus follows up *B. influenzæ* very readily. Fildes, Baker and Thompson (1918) found that in the fatal broncho-pneumonias of the influenza epidemic then prevalent, pneumococci were commonly found in the centre of pulmonary lesions, while *B. influenzæ* only was detected in their spreading margins. Malloch (1922) observed an association of *B. influenzæ* with a majority of the cases of broncho-pneumonia in which he isolated pneumococci. In adults at all events, therefore, there is some reason for supposing that the pneumococcus in so far as it is concerned with broncho-pneumonia is a secondary invader or an associated infection, the surfaces of the respiratory tract being possibly opened to the attack of pneumococcal strains of lesser virulence on account of the damage that they have sustained from *B. influenzæ*. Such a supposition is put forward very definitely by Hübschmann (1915), who advances evidence for the production of lesions of the finer bronchioles during an attack of influenza, which render the lung peculiarly liable to invasion by other bacteria, notably the pneumococcus.

It is possible that a similar explanation may underlie the frequency of broncho-pneumonia in childhood. For in the conditions most often associated with that disease, such as measles and whooping-cough, the extent to which the respiratory tract may be overrun by bacilli similar to or identical with *B. influenzæ* is well recognized (Bordet and Gengou, 1906; Wollstein, 1906; and Davis, 1915). And although in examinations

of the bacteriology of broncho-pneumonia in childhood, such as that of Lyon (1922), pneumococci have been found more frequently than *B. influenzæ*, the readiness with which the latter may be dominated by the former must be taken into account. Meunier (1919) pointed out that in cases of influenzal broncho-pneumonia, *B. influenzæ* at first predominant in the bronchial secretions would be swamped by pneumococci or *M. catarrhalis* after the fourth day. In Glynn and Digby's (1923) series of pneumonias there were 22 broncho-pneumonias and the only pneumococcus found in the sputum of 16 of these was a Group IV organism. Four cases examined by lung puncture yielded, one, a Group IV pneumococcus ; one, a mixed infection of Group IV pneumococci and *Staphylococcus aureus ;* one, *B. influenzæ ;* and one, meningococcus. That is to say that their most reliable observations on the bacteriology of broncho-pneumonia showed a bacteriology as diverse as that observed in the crop of pulmonary lesions which appeared at the end of the recent influenza pandemic.

There are fewer data available with regard to the effect of other bacteria upon the pneumococcus. It does not appear often to invade the body successfully in combination with the staphylococcus, which is not surprising in view of the markedly inhibitory action which it produces on that bacterium (McLeod and Govenlock, 1921 ; Alivisatos, 1925). The association of streptococci of the hæmolytic type and pneumococci in the irregular types of pneumonia of the 1918 influenza pandemic was not infrequent. Such conditions have been described in considerable detail by MacCallum (1919). In a group of casesin which interstitial and lobular pneumonias predominated over lobar forms in the ratio of 4 to 1, hæmolytic streptococci were found in 82 per cent., *B. influenzæ* in 47 per cent., and pneumococci chiefly in cases with a tendency to lobar consolidation. Cole and MacCallum (1918) describe observations on cases of this type which suggest that in such lesions the streptococcus tends to overrun the pneumococcus. In a large series of cases of lobar pneumonia Baermann (1914) observed the following mixed infections : pneumococcus with *B. typhosus*, 5 cases ; with staphylococcus, 6 cases, all of which recovered ; with streptococcus, 3 cases, all of which died.

There has been much diversity of opinion with regard to the influence which secondary infection may have on the evolution of pulmonary tuberculosis. Investigations with regard to the significance of the pneumococci in so far as that can be determined by discovering the percentage of tuberculous sputa containing pneumococci, and the type and virulence of the organism found, have been made by Lyall (1915) and Corper, Donald and Antz (1919) amongst others. These investigators found the predominant types of pneumococcus in tuberculous sputa were those most often found in the normal mouth, and further, that the severity of the disease was equally independent of the presence of pneumococcal types of high pathogenicity in the sputum and of the presence of pneumococci of any kind.

Progress of the pneumonic lesion and natural resistance.

The histological features of pneumonia in its various stages are too well known to require description : but it is desirable to emphasize certain features of the lesion which bear on the ultimate fate of the invading micro-organism or are related to the special type of pneumococcus responsible for the lesion.

Variations in lesion according to type of pneumococcus responsible for the infection. The methods of differentiating the various pneumococcal types are described on p. 204, and need not be further considered here. It is stated by Lamar and Meltzer (1912) that pneumonias due to Type III pneumococci are associated with an exudate which is particularly moist and viscid, and according to Cecil, Baldwin and Larsen (1927) empyema is almost twice as frequent in pneumonia due to Type I pneumococci as in any other.

Circulation in the pneumonic lung. Gross (1919), who studied the condition of the circulation in the viscera *post mortem* by irrigating with a suspension of barium in gelatin and taking X-ray photographs, subsequently, gathered the impression that there was a very marked ischæmia of the consolidated portions. More recently Christie, Ehrich and Binger (1928) have got evidence for the same conclusion by observations on the lungs of living dogs in which consolidation had been produced by insufflation of cultures of Friedländer's bacillus. Their method was to observe by thermo-couples whether the consolidated part showed a rise of temperature under diathermy in the same way that a lung did in which the circulation was artificially obstructed. This was found to be the case, whereas the normal lung with unimpeded circulation showed very slight rise of temperature under the same conditions. It would appear, therefore, that the pneumonic lesion is to a considerable extent shut out of the circulation. The chief factors producing this result are probably pressure on the capillaries of the alveolar wall on account of distension of the alveoli by exudate, and also sometimes the formation of thrombi obstructing many of the capillaries.

Observations by direct puncture of the lung on the behaviour of the pneumococci within the consolidated areas. This method has been adopted by a number of observers—amongst others by Rosenow (1911[1]). His chief observations were that from the outset the chief cell in the exudate was the polymorphonuclear leucocyte ; that in cases proceeding to crisis and recovery there was a gradual or in some cases sudden disappearance of the pneumococci ; whereas in those in which death followed there was a steady increase in the numbers of pneumococci present. Phagocytosis of well-preserved pneumococci was not observed : it only occurred where numerous degenerating cocci were seen in the fluid. Thomas and Parker (1920) in discussing observations of this kind state that in most pneumonias proceeding to recovery pneumococci are not recognized in puncture fluids taken after the fifth day : that is, they often disappear some time before the actual clinical crisis. In Glynn and Digby's (1923) series of cases

examined by this method positive results were obtained in about 50 per cent., and seemed, like positive blood cultures, to indicate a more grave prognosis.

The Nature of Crisis and the Parts played in it respectively by the Natural Defensive Forces and those Acquired in Response to the Presence of the Pneumococcus in the Lesions.

The principal question at issue is : what is responsible for the destruction of the pneumococci in the lung in those cases in which recovery takes place either by crisis or by lysis ? Are the leucocytes and interactions between them and the pneumococci responsible without intervention of antibodies, or do antitoxins, bacteriotropins and bactericidal immune bodies generated on account of the presence of the pneumococci in the body play the chief part ? Neufeld and Händel (1913), in reviewing the evidence then available on the subject, concluded that there was no sufficient ground for supposing that antitoxin plays a part in recovery, and they could not convince themselves that a flooding of the circulation with bacteriotropic and other antibodies at the time of crisis had been clearly demonstrated. They were inclined to think that the natural bactericidal agents of the body, particularly those derived from the leucocytes, might be important. This general survey of the earlier literature on the subject seems to the writer a sound one, and only observations made at the same time or subsequently need be further considered.

Influence of toxin and antitoxin. It cannot be said that the observations of the last fifteen years have clearly shown that antitoxin plays a part in crisis. Cole (1914[1]) described a hæmolytic toxin affecting the guinea-pig as a general toxin, but he could not demonstrate antitoxin formation in human sera during or after pneumonia. Lister (1916) pointed out that a man readily tolerates 40,000 million dead pneumococci intravenously or subcutaneously, and this disposes of the idea that endotoxin is important. Further, Cole (1914[2]) could get no evidence of toxic effect when the blood of a rabbit heavily infected with pneumococcus was filtered just before its death, and injected to a second rabbit intravenously. Chesney and Hodges (1922) state that most experiments demonstrating toxic effects with pneumococcal products have been unreliable as they have depended on anaphylactoid phenomena following intravenous injections. Their own attempts to elicit toxic effects by injections of filtrates to the peritoneum of the mouse in amounts of 1 c.cm. have invariably failed. There remains the possibility that the soluble specific substance acts as toxin ; this is discussed in detail on pp. 214, 215 ; but it must be remembered that though it can act as aggressin (Sia, 1924–5), there is no direct evidence that it is a general toxin (Felton and Bailey, 1926).

Influence of the bactericidal action of serum. Since there is agreement amongst different observers (Dold, 1911 ; Dold and Muff, 1911 ; Barber, 1919 ; Bull and Bartual, 1920) that distinct bactericidal activity is absent equally in normal and immune serum, such action need

not be considered in explanation of crisis, Longcope (1905) even found that pneumococci grew more freely in many pneumonic sera than in normal sera.

Influence of agglutinins and precipitins. Although these substances have no direct bactericidal action they may be important as part of the mechanism of recovery. It was shown in the experimental work of Blake and Cecil (1920[1 & 2]) on monkeys that the pulmonary lesion may heal by crisis while septicæmia progresses ; agglutinins may therefore play an important part in recovery by checking septicæmia, while precipitins by neutralizing soluble specific substance (aggressin) may facilitate phagocytosis, i.e. act as bacteriotropin. The fact that something very like crisis occurs in pneumococcal septicæmia in the dog (Bull, 1916), i.e. steady increase of circulating pneumococci up till the fourth or sixth day, and then their sudden disappearance, might be explained by development of agglutinins, and it is suggested by Bull and McKee (1922) that agglutination of pneumococci on the walls of the capillaries takes place before demonstrable agglutinins can be detected in the circulating blood. It is possible that the protective qualities which may be demonstrated in pneumonic sera by animal experiments are due to precipitins and agglutinins. Such qualities were noted by Armstrong (1925) as appearing about the fifth to sixth day of disease ; he did not observe them in normals, and Baldwin and Rhoades (1925) have shown that the appearance of such substances in the sera coincides with the cessation of bacteriæmia. Clough (1924), however, states that protective qualities may be present in the sera of normal individuals.

Influence of leucocytes and of leucocytic extracts and of other phagocytes. Influence of opsonins and bacteriotropins. There are good reasons for supposing that the polymorphonuclear leucocyte plays an important part in the recovery from pneumonia. Cross (1915) found that amongst 54 cases with a leucocytosis below 15,000, there were 30 per cent. which developed crisis and 26 per cent. which died. While in a similar number of cases with a leucocytosis above 20,000, there were 40 per cent. which developed crisis and only 17 per cent. which died. Similar data were obtained by Avery, Chickering, Cole and Dochez (1917) in more extensive observations. Clough (1917) studied the variations in leucocytosis according to type of pneumococcus responsible for the pneumonia and found that in Type III pneumonias, which are generally recognized as the most dangerous and were all fatal in her series, the number of leucocytes in the blood was half or less than half as many as were found in pneumonias due to the other types. Blake and Cecil (1920[2]) found that leucocytosis fell when blood invasion became pronounced in experimental pneumococcal infections in the monkey, and that degenerative changes of the myeloid tissues might also occur.

In contrast to the absence of evidence of bactericidal activity in serum, plasma, &c., there is a good deal of direct experimental evidence for a bactericidal action which may be exerted by leucocytes and leucocytic

extracts. Bogendorfer (1921) found that pneumococci were killed in suspensions of leucocytes in blood or active serum, whereas streptococci were not. Dold (1911) observed a bactericidal effect on pneumococci when leucocytic extracts were used, which was independent of the virulence of the strain, and he attributed the slight bactericidal action present in human plasma and absent in that of mice and rabbits to secretion of the leucocytic bactericidal substances to the tissue fluids in man. Wright (1927) has shown that the bactericidal action for pneumococci which can be demonstrated in the whole blood of an immunized rabbit is definitely connected with the cells, although in these experiments he does not attempt to differentiate between red and white cells. His experiments in the rabbit showed that the polymorphonuclear leucocytes played an important part in the phagocytosis of pneumococcus in the lungs, but that in the liver the monocytes, including apparently the Kupffer cells, played a part as great or even greater.

Since the leucocytes appear to play an important part in recovery from pneumococcal infection the study of substances of the nature of opsonins and bacteriotropins is also important. It is shown below (pp. 215, 216) that substances corresponding to bacteriotropins are the chief antibacterial bodies present in immune sera, and may actually correspond to precipitin for specific soluble substance. The records of observations on variation in the opsonic activity of the serum during pneumonia were mostly published fifteen or more years ago, and yielded variable results (Macdonald, 1906; Strouse, 1911; Tunnicliff, 1911; Clough, 1912; Lister, 1913). Wadsworth (1912) even maintained that the occurrence of phagocytosis was uncommon in human lesions due to pneumococci. Robertson and Sia (1927), however, show that if the opsonic technique is varied by treating small quantities of bacteria with excess of serum the fact is revealed that all animals resistant to pneumococcal infection possess natural opsonin, whereas all susceptible animals lack it. It would seem possible, therefore, that the inconsistent results obtained in observing variations of opsonic activity in the course of pneumonia are due to defects in the original opsonic technique, and that the problem may be illuminated by repeating this work with the modified method of Robertson and Sia.

Possible influence of the character of the metabolism of the pneumococcus on crisis. Observations of McLeod and Govenlock (1921) showed that when certain strains of pneumococci were cultivated continuously in tubes of serum broth, and the number of living bacteria estimated by daily subculture, it was seen that at intervals of 2 to 3 days the living cocci were reduced to very small numbers, a loopful often giving no growth, whereas a moderately profuse growth could be obtained on the following day. Such observations suggest the possibility that the bacteria themselves may be partly responsible for the peculiar tendency to crisis characteristic of pneumococcal infections.

That the pneumococcus will die off in culture media containing glucose on account of the acid which it produces is well recognized (Avery and Cullen, 1919 ; Lord and Nye, 1919). Lord (1919) maintains that there is a definitely acid reaction in the press juice of the pneumonic lung, and he suggests the possible influence of this on crisis. He found that in a dog, the subject of experimental pneumonia, the pH of the press juice from one area involved was 5·4, and from it no pneumococci could be grown, whereas in another area involved the pH was 6, and cultures of pneumococci were obtained. Another possibility is that the H_2O_2 formed by the pneumococcus plays some part. It is altogether probable that H_2O_2 is produced in the pneumonic lung. The production of methæmoglobin in pneumonia is assumed by Peabody (1913) on account of the dark brown colour of the blood developed in severe cases, and its defective powers of oxygen absorption, and Schnabel (1921) has demonstrated methæmoglobin bands in the blood of mice heavily infected with pneumococci. The mechanism by which the pneumococcus forms methæmoglobin is intimately associated with that by which it produces H_2O_2 since Neill and Avery (1924[1 & 2]) have shown that the same pneumococcus extracts which have marked powers of producing methæmoglobin from oxyhæmoglobin give rise to H_2O_2 when exposed to oxygen. It is therefore obvious that when the pneumococcus is producing methæmoglobin in the presence of oxygen it will also produce H_2O_2. It is also shown in the work referred to that the oxidizing powers of the pneumococcus exceed those of any amount of H_2O_2 which it is likely to form, and are exercised independently of catalase, which, of course, must be present in large amounts in the pneumonic exudate. It is to this marked oxidizing effect that the bleaching of blood-pigment characteristic of the pneumonic lung in areas of grey hepatization is most probably due. Whether such oxidative effects play any part in the death of the pneumococcus in the lung is far from clear ; they may well do so, however, for in the experiments of Phelon, Duthie and McLeod (1927) it was shown that the pneumococcus dies off rapidly in blood broth cultures when these are vigorously oxygenated, notwithstanding the presence of abundance of catalase. There is a marked discoloration of the blood in such cultures which does not occur in those of bacteria incapable of H_2O_2 production.

The present state of our knowledge with regard to the cause of the death of the pneumococcus about the time of crisis may be summed up as follows : (1) There is no evidence that accumulation of antitoxin is responsible for crisis ; (2) the production of agglutinins, precipitins and bacteriotropins may and probably do play a considerable part in controlling general invasion of the body by the pneumococcus ; (3) since the pneumococcus is dying off in the consolidated area in cases evolving favourably before any considerable concentration of such substances can be demonstrated, and since they have never been demonstrated in some cases, it is unlikely that they explain the death of the pneumococci in the consolidated area of the lung ; (4) the action of bactericidal substances

derived from the leucocytes and possibly aided in some way by the products of bacterial growth—acid and peroxide—are most probably responsible for the death of the pneumococci within the consolidated area.

Resolution.

In resolution the exudate, which is practically blood-clot rich in leucocytes, is dissolved and absorbed, whereas intravascular clot is usually organized. It seems likely that the ferments liberated from the disintegrating leucocytes are responsible. Nye (1922) showed that the cellular exudate from pneumonic areas had considerable powers of dissolving fibrin at a pH range 6 to 8, whereas the proteolytic enzymes which may be obtained from the cells of normal lungs are less active, and that only when the reaction is much more acid. Lord and Nye (1921) describe the presence of two enzymes in the pneumonic lung, one proteolytic like that described above, and the second peptolytic with maximal activity at pH 6·3 to 5·2, and they suppose that the acid reaction of the pneumonic area brings these into operation in succession and so resolution occurs. They further show that exudate from areas of grey hepatization erodes Löffler's serum, but that that from areas of red hepatization only does so if first washed.

Jobling, Petersen and Eggstein (1915) suggest that fever and intoxication in pneumonia are mainly due to absorption of proteose (disintegrated fibrin) from the lesion, and that crisis and resolution are simultaneous because the protein being digested beyond the proteose stage is no longer toxic. The factors likely to promote resolution in their view would be (1) acidity reducing the dispersion of antiferment, (2) oxidation of the unsaturated linkages in the antiferment, (3) decrease in serum antitrypsin. Their own observations showed that a decrease of serum antitrypsin was the rule at the time of crisis, and from what has been said above it can be seen that by producing an acid reaction and initiating active processes of oxidation the presence of the pneumococci in the exudate may promote the conditions tending to crisis and resolution if the explanation of the phenomena advanced by these authors is correct. Whether the slight protein-splitting powers of the pneumococcus demonstrated by Foster (1913) and Avery and Cullen (1920[1]) play any part in resolution is not clear. It is probable that the small amount of blood (Kline, 1917) which passes through consolidated areas of the lung is only sufficient to control tissue reaction and inhibit proteolytic processes in the structural framework. The general suggestion from these observations is that the stage of grey hepatization is essential to good resolution, and that organization will follow when the process does not go beyond red hepatization.

Relapse and Recurrent Pneumonia.

Bungart (1908), who defines relapse as reappearance of frank consolidation and fever after complete clearing, and absence of fever for a sufficient period to eliminate false crisis, only observed 3 definite cases in a series of 500 pneumonias, but in 2 of these he followed the case

throughout and confirmed the preliminary resolution by X-ray examination. All these recovered. Baermann (1914) observed the phenomenon more frequently as there were 2 to 3 per cent. in his series of over 500 pneumonias ; all terminated in recovery. Kohn (1925) quotes extensive statistics from the literature to show that in nearly 20 per cent. of cases pneumonia is a recurrent infection, and he describes two attacks of pneumonia within five weeks in a patient with a previous history of repeated attacks. In both the infections observed the pneumococcus isolated was a Type I organism : the patient recovered.

The general deduction suggested by these observations is that a high and permanent immunity is not developed to the pneumococcus, and they strengthen the conclusion that factors other than those of acquired immunity play a considerable part in recovery from pneumonia, while the fact that recovery after relapse is the rule suggests that the existence of some acquired general immunity prevents the development of extensive pneumococcal invasion of the body.

Causes of Death in Pneumonia.

Some causes of death, such as failure of the heart to cope with the increased demand on it, are obvious, but there are two possible factors more directly associated with the pneumococcus to be considered, namely, the development of an overwhelming septicæmia and the existence of exceptionally toxic strains or of individuals peculiarly susceptible to toxic substances which the pneumococcus produces. Baermann (1914) describes two cases in his series in which the patient died although pneumococci were never isolated from the blood and only a small area of lung was found consolidated *post mortem*. It is difficult to explain such cases except on the ground of toxic effect, but until some convincing demonstration that the pneumococcus does produce toxin is forthcoming such an explanation must remain hypothetical. In connection with such cases Lamar and Meltzer's (1912) observation, that there were only three of their dogs which died from experimental pneumonia without developing septicæmia, and that two of these had necrotic foci containing sporing anaerobes in their lungs, is suggestive.

There is a general consensus of opinion that any considerable degree of septicæmia has a grave prognostic outlook. Baermann (1914) had only 2 recoveries amongst 35 patients in whose blood pneumococci were numerous, and Sutton and Sevier (1917) found in a small series of cases of pneumonia observed by daily blood culture that with one exception all patients whose blood yielded at any time more than six colonies of pneumococci per c.cm. died. Both Cole (1914[2]) and Schottmüller (1905) have observed cases in which the pneumococci have been present in the blood at the time of death in very large numbers. Even when a massive septicæmia does occur the cause of death remains obscure ; although a possible explanation may be that by methæmoglobin formation or in some other way the pneumococcus hampers intimate tissue respiration.

Other Lesions due to the Pneumococcus.

Acute otitis media and meningitis. According to Bleyl (1914) acute otitis is commonly caused by capsulated bacteria—the pneumococcus, *Streptococcus mucosus* and *B. mucosus capsulatus*—and the first is associated with the most benign types, in which the fever is slight and the discharge dries rapidly. Some cases of pneumococcal meningitis are due to extension from such lesions, but others occur as a sequel to fracture of the base, and it appears from the work of the French that meningitis is a rather frequent complication of acute general pneumococcal infections in specially susceptible races, and may closely resemble in its clinical aspects a fulminant meningococcal infection (Dufougère, 1914; Cotoni, Truche and Raphael, 1922). Primary meningitis is not particularly uncommon in this country, however, since Worster-Drought and Kennedy (1917) observed 5 infections which appeared to be primary in a series of 9 cases. On 3 of these an autopsy was performed, and no lesion but the meningeal one could be detected. It is very constantly fatal, but an undoubted case of recovery has been recorded by Campbell (1925).

Corneal ulceration. The association of the pneumococcus with serpiginous corneal ulceration is well recognized and Cramer (1915) states that in his experience it is invariably preceded by a latent pneumococcal infection of the tear sac.

Vulvovaginitis, peritonitis, appendicitis and hepatitis. Bryant (1901), reviewing the literature of pneumococcal peritonitis, noted 43 cases in girls and only 9 in boys, and gathered the impression that the lesion was usually a primary one. Bondy (1912) examining the vaginal secretions of 90 women found the pneumococcus twice, and concluded from his own findings and records in the literature that this bacterium might be responsible for genital infections in the female. In the experience of the writer the pneumococcus has been obtained in pure culture from the pus associated with vulvovaginitis. McCartney and Fraser (1922) subjected a series of 56 cases of pneumococcal peritonitis in young children to careful examination. Amongst 20 cases which were presumably secondary—a pneumococcal lesion being found elsewhere in the body—there were more cases amongst boys than amongst girls. But the remaining 36 cases, all primary, occurred in the female. These facts, together with the observation that the infection appears to spread upwards from the pelvis, strongly suggest that a primary pneumococcal peritonitis is a disease of young girls, and is chiefly due to an upward infection of the genital tract as soon as it becomes patent and as long as the secretions are alkaline, i.e. before puberty. In a series of 50 cases of appendicitis, Isabolinsky (1914) found streptococci in 7 and in 6 others a diplococcus pathogenic to mice, which he assumed to be a pneumococcus. These 13 cases were the ones in which lesions in the appendix were most marked, and they were associated with sore throats.

Lemierre and Abrami (1910) observed three fatal cases of pneumonia complicated by jaundice and recovered the pneumococci in pure culture from the gall-bladder in each case. Since the bile-passages were patent they concluded that the jaundice was due to hepatitis, and they suggest that some cases of jaundice may be sequels to general pneumococcal infections in which the lung is not involved. A somewhat similar idea is developed by Calmette, Vansteenberghe and Grysez (1906) who found that pneumococci administered to rabbits and guinea-pigs by stomach-tube reached the lungs in considerable numbers, but never caused pneumonia, although the animals were usually ill for a few days. They suggest that many of the unexplained febrile disturbances of childhood may be due to bacteria taken into the system in this way before immunity has been developed.

Empyema, pericarditis and endocarditis. The experience gained in doing the bacteriological work of any general hospital shows clearly that the pneumococcus is much the most common cause of empyema. It is not clear why it arises in connection with some pneumonias and not in others. Thomas and O'Hara (1920) observed 60 per cent. of Type I infections in a series of 30 empyemata. And Malloch (1922) and Lyon (1922) found also that this condition was most often produced by pneumococcus Type I. Glynn and Digby (1923), however, examining a series of 30 cases of empyema in children, found Group IV to occur most frequently and to be associated with the graver cases. Empyema differs in its progress from pneumonia, since it may proceed independently of crisis, and is possibly due to a lymphatic spread. Pericarditis is rarely seen except in conjunction with extensive general involvement of the body.

In Wright's (1925) series of blood cultures on 29 cases of subacute infective endocarditis the pneumococcus was only found three times, and each time within five days of death in patients who at autopsy were discovered to exhibit other pneumococcal lesions. This is in keeping with the general experience that pneumococcal endocarditis is a terminal event in the course of a general pneumococcal infection. Very large vegetations are characteristic of this condition. Baermann (1914), however, records that one patient from whom pneumococci were obtained in blood culture for four months after recovery from pneumonia had occasional bouts of fever, and, although dying from some other cause, showed the lesions of chronic ulcerative endocarditis at autopsy.

Bacteriological Diagnosis.

By J. W. McLeod.

Bacteriological diagnosis is often a relatively simple matter. The smooth slightly raised translucent colony which appears on fresh blood-agar or heated blood-agar together with the green colour which is developed make the recognition of colonies easy. The central pitting which occurs in many colonies of this type after 18 hours' incubation is highly

characteristic. These appearances, together with the demonstration of bile-solubility by the methods already described, are adequate for the identification of the pneumococcus. The demonstration that high dilutions of broth cultures of strains just isolated are pathogenic to mice can be used as additional evidence. For isolation of pneumococci from the throat Gordon (1921) found that the best method was inoculation of 5 per cent. blood dextrose broth from a swab and subsequent inoculation to the peritoneum of mouse of 1 c.cm. of the culture after 8 to 12 hours' incubation. The methods of type identification are described on p. 204. An additional method of diagnosis is the demonstration of soluble specific substance in various body fluids such as pleural exudate, serum or urine by observing precipitate formation when these are mixed with the corresponding antisera (Dochez and Avery, 1917; Cole, 1917).

It is noteworthy that pus from empyema fluids which shows numerous degenerate pneumococci when examined under the microscope may yield no growth when cultivated on suitable media. Bolognesi (1907) observed a case of this kind in which he was only able to obtain growths under anaerobic conditions at the outset.

Gutfeld and Nassau (1926) found that 90 per cent. of young children gave a skin reaction on injection of pneumococci, but that during and for some time after broncho-pneumonia this reaction disappeared.

Spread of Infection.

By J. W. McLeod.

A micro-organism so frequently the inhabitant of the normal or approximately normal throat as the pneumococcus may well invade the tissues when their resistance is weakened by fatigue and exposure. It has been suggested, however, that an important part in the incidence of pneumonia is played by certain strains of pneumococcus of special virulence, which pass from person to person, causing pneumonia in some and harboured by others without injury, but rendering them dangerous to their neighbours as carriers of disease. This idea has been specially elaborated in America. It is claimed by Avery, Chickering, Cole and Dochez (1917) and by Cole (1918) that Types I and II pneumococci are to be regarded as strains peculiarly liable to cause pneumonia, since they are rarely present in the mouth and throat, except in those who are recovering from an attack of pneumonia or are contacts of pneumonia cases, whereas Type III and Group IV pneumococci since they are commonly present in the mouth have less significance. It is suggested further that by isolating carriers of the former types the ' epidemic ' spread of pneumonia may be cut down, and that the latter types are responsible for the sporadic pneumonias consequent on exposure, &c., and depending on auto-infection. Some of the data advanced by these authors in support of this thesis are given in Table I.

TABLE I.

Observers.	Source of Cultures and Numbers of Investigations.	Incidence of Types, Per Cent.				
		Type I.	Type II.	Type II (Atypical)	Type III.	Group IV.
Avery, Chickering, Cole and Dochez.	Saliva of normal men: 297 examined; 116 yielded cultures of pneumococcus ..	0·8	0·0	18·2	28·1	52·9
	Room dust, ordinary: 62 specimens examined; 18 specimens + ..	5·5	0·0	38·6	11·0	44·4
	Room dust from pneumonia wards: 183 specimens; 74 + ...	33·8	31·1	5·4	2·7	27·0
	Saliva of contacts of Type I pneumonia, 160 ..	13·1	—	—	—	—
	Saliva of contacts of Type II pneumonia, 149 ..	—	12·1	—	—	—

Considerable and quite independent support for this point of view comes from work of Baermann (1914) carried out before the type differentiation amongst pneumococci was generally recognized. This writer, surveying more than 500 cases of pneumonia which occurred on a Javan plantation amongst 18,000 labourers, came to the conclusion that three factors operated, (1) small localized epidemics, limited to groups of labourers in one hut or otherwise closely associated, and independent of weather conditions since these were similar all over the plantation. In such groups of cases there was usually considerable similarity of clinical features which differed from one outbreak to another. These outbreaks he thought depended on the passage of strains of special virulence from man to man; (2) a generally increased incidence of pneumonia all over the plantation at certain seasons and dependent on weather conditions; (3) a small stream of hospital infections of rather severe course, which he assumed to be due to a definite type of pneumococcus more prone than others to persist in the wards.

The answer to the question ' what are the weather conditions which promote pneumonia ? ' has been most convincingly presented by Rogers (1925) who bases his argument on the incidence of pneumonia in the prison population all over India—1,500,000—during a period of 14 years. He found a gradation in incidence of pneumonia from 50 per 1,000 in the North-West to 8 per 1,000 in the South-East. And comparing this incidence with the prevailing weather conditions he concludes that the occurrence of pneumonia is promoted by (1) a wide range of temperature in the course of the 24 hours, (2) a low maximum temperature in the cold season and, (3) a low atmospheric humidity. It is interesting in this connection that Richter (1911), analysing the records of climatic conditions and pulmonary disease during the period in which accurate records are available, found that the long periods of low atmospheric humidity (anticylonic conditions) corresponded to periods of high incidence of acute pulmonary inflammations. The inference that Rogers draws from his data is that chill is the most important element in producing pneumonia.

Cecil (1921) states that during influenza epidemics the type incidence changes so that Group IV strains predominate. This enhances the importance of such strains, since it is during influenza epidemics that the most serious mortality from pneumonic conditions arises. The possibility that variation from type to type (Griffith, 1928) may occur makes it necessary to reserve judgment as to the paramount importance of pneumococci of Types I and II in the spread of pneumonia.

Another aspect of the subject is developed by Lenz (1917) who assumes the development of the condition of pneumococcal carriers in a large proportion of a community as a result of epidemic catarrh, and the succumbing of a small number of these to pneumonia. Neufeld (1922) makes a similar suggestion, supposing that the incidence of pneumonia may resemble that of meningococcal meningitis, in which the disease appears in a small percentage of susceptible individuals as soon as a high percentage of the community become infected with the micro-organism, while case to case infection plays a very secondary role.

Although the pneumococcus would appear from the French writing to be particularly dangerous to some of the troops from North Africa, Kelly (1926) on collecting large statistics did not find that the negroes of America were more susceptible to pneumonia than white men, and Rogers (1925) attributes the higher incidence in native Indians to the fact that they are much more poorly clad than the Europeans, and hence more exposed to chilling. It is probable that the apparently high racial susceptibility occasionally described depends on the introduction of a race accustomed to conditions unsuitable for the development of pneumonia to climatic and social surroundings which favour that disease.

Serological Races of Pneumococci.

By F. Griffith.

Significance of Types.

Classification.

The American classification of pneumococci, based on agglutination, precipitin reactions and protection tests, is now accepted in the majority of countries, and the strains and sera of the Rockefeller Institute have been adopted as standards. From the results of their earlier studies, based on the pioneer work of Neufeld, the American investigators (Avery, Chickering, Cole and Dochez, 1917) found that pneumococci in lobar pneumonia fell into two general classes. The larger class contained three well-defined types which they designated I, II and III, the last being the *Pneumococcus mucosus*. The smaller class which included all the strains not agglutinated by any of the three chief type sera they termed Type IV ; this was a tentative designation and has been superseded by the term Group IV. Originally this group was supposed to consist of strains all of which differed serologically from each other as well as from Types I, II and III, but M. Olmstead (1917) has succeeded in classifying 94 strains of this heterogeneous group into 12 serological types ; similar evidence of grouping has been obtained by Stillman (1919), Griffith (1922), Christensen (1923) and others, but the number of serological differences encountered has been found too great to permit of an exhaustive classification. There remains the question of certain strains which Avery (1915) designated ' atypical Type II ' because they gave some response with concentrated Type II serum, though not up to the titre of the fully authenticated Type II cultures. While there is no doubt of the immunological relationship of these atypical strains to the chief Type II, it is equally clear that each possesses a specific antigen which enables it to be classified as a distinct type. Whether the evidence afforded by their precipitin formation with the serum of Type II is sufficient to justify their classification as variants of that type cannot be decided until the larger question as to possible variations in antigenic relationship between one type and another is settled. At present they should be regarded as members of Group IV.

In regard to the designation of type employed prior to the American classification, it has been shown that the American Type I corresponds to the typical strain originally isolated by Neufeld in Germany, while one of the latter's atypical strains conforms to the standard Type II of the Rockefeller Institute. In South Africa, Lister's Groups C, B and E correspond respectively with the American I, II and III, while the numerically most important South African group, viz. A, together with G, J and K, would, according to the American classification, be relegated to the heterogeneous Group IV. Lister's A pneumococcus was obtained in London by Urquhart (1922) five times in his series, and he classified it on the grounds mentioned above as a sub-group of Type II.

Lobar pneumonia. It is a fact of considerable interest from the epidemiological standpoint that the incidence of the chief types of pneumococci in lobar pneumonia is so nearly the same in countries with similar climatic and social conditions. Lister's figures for South Africa diverge to some extent from those obtained in America and Great Britain, the chief distinction being that the type causing by far the largest number of cases of pneumonia in Lister's series has not shown the same tendency to spread in the latter countries. The explanation of the different incidence of the types is no doubt to be found in the special circumstance that the population dealt with by Lister was composed of native workers in the mines.

Though variations are observed from year to year in different districts of the same country, and in the one country as compared with the other, the following figures represent the approximate proportions for the various types occurring in lobar pneumonia in Great Britain and America, viz. Type I = 30 to 40 per cent., Type II = 20 to 30 per cent., Type III = 0 to 16 per cent. and Group IV = about 30 per cent. Glynn and Digby (1923) have analysed and tabulated the results of various researches on the incidence of the pneumococcal types in Britain and America. More recent American figures are given by Cecil, Baldwin and Larsen (1927), and the subject has been investigated by Malone (1923, 1924) in India, Christensen (1923) in Denmark and Thjøtta and Hanneborg (1924) in Norway. In Germany the different types have been identified, but their distribution has not received so much attention as in some other countries. Hintze (1926) showed that Type I (Neufeld's typical pneumococcus) was present in 25 per cent. of his cases of typical pneumonia; the author was less confident in regard to the classification of his remaining strains. Lister (1917) in South Africa classified 148 strains of pneumococci from cases of pneumonia as follows: 21·6 per cent. in Group C (Type I), 16·2 per cent. in Group B (Type II), and 2 out of 148 in Group E (Type III). Among the remaining strains which, according to the present method of classification, would fall into Group IV, Lister identified 9 different groups, including Group A (31 per cent. of the whole). The French investigators (Cotoni, Truche and Raphael, 1922) have adopted different methods and the results of their classification are not comparable with those cited above. The fact that they did not confirm the sharp demarcation between the different types may, to some extent, be attributable to the method employed for the preparation of the agglutinating sera. The pneumococci were grown for 15 to 18 hours in broth and then centrifuged; the deposit from the cultures was treated with alcohol-ether, dried *in vacuo* and rubbed into a very fine powder. A serum prepared with such an inoculum would probably contain a considerable proportion of antibodies to the protein substance of the pneumococcus which is apparently common to all pneumococci irrespective of type (see p. 173).

Table II gives the distribution of the types found in various investigations.

TABLE II.

Types in Lobar Pneumonia.

Author.	Country.	Total Cases.	Percentage Incidence of Types.			
			Type I.	Type II.	Type III.	Group IV.
Glynn, Digby and Jones.. ..	America	1,632	32·5	22·8	11·6	33·1
,, ,, ,, ...	Britain	361	38·2	26·6	3·9	31·3
Cecil, Baldwin and Larsen ..	America	1,913	33·6	19·7	13·3	33·1
Malone	India	106	28·3	17·0	7·5	47·2
Christensen	Denmark	110	33·0	27·0	8·0	32·0
Thjøtta and Hanneborg	Norway	100	44·0	33·0	6·0	17·0
Hintze	Germany	134	25·0	3·7	6·7	—

In *broncho-pneumonia* there is a much greater irregularity in the incidence of the pneumococcal types, and the preponderating part is played by the pneumococci of Group IV, which are found in roughly 75 per cent. of the total cases. McClelland (1919) in America examined 723 cases of post-influenzal pneumonia, and found Type I in 1·1 per cent., Type II in 8·4 per cent., Type III in 11·6 per cent. and Type IV in 78·9 per cent. In England, out of 21 cases, Glynn and Digby (1923) found 4 examples of Type II, the rest belonging to Group IV; Malloch (1922) examined 20 cases and found Type I twice, Type II five times and Type III twice, the remaining 11 strains belonging to Group IV.

In the *normal mouth* Group IV pneumococci occur most frequently. The workers of the Rockefeller Institute examined the mouth secretions of 297 persons who gave no history of contact with an acute or recent case of lobar pneumonia and obtained pneumococci from 116. Analysis of these strains by serological methods revealed only one example of Type I and none of Type II; Type III was found 34 times, a percentage incidence of 28·1; the remainder which were identified according to the American nomenclature as representatives of the various sub-groups of Type II and of Type IV belonged to Group IV. Glynn and Digby studied the pneumococci obtained from the saliva of 25 normal persons and found that all belonged to Group IV.

Type in Relation to Mortality.

The first observations on the relation between serological type and death-rate in lobar pneumonia were made at the Hospital of the Rockefeller Institute. The figures given by Avery, Chickering, Cole and Dochez (1917) show that Type IV was the least virulent and that Type III gave rise to the highest mortality. On the other hand, Cecil, Baldwin and

Larsen (1927), from an analysis of 1,107 cases of pneumonia treated at the Bellevue Hospital, New York, in which no specific treatment was given, showed that the death-rate from Type I pneumonia was lower than that of any of the other types. The percentage mortality was 20·7 for Type I, 42 for Type II, 41·6 for Type III and 29·2 for Type IV. The low mortality in Type I pneumonia is attributable to infection with this type being most common in young people, in whom the prognosis is known to be favourable. The fact that the elderly and patients with chronic systemic disease are particularly liable to Type III pneumonia accounts for the high death-rate under that type; in the young and healthy Type III pneumonia is not specially severe. The mortality rate in Type IV pneumonia varies with the kind of patient, and is highest when it occurs in association with influenza and as a complication of various chronic diseases.

Technique of Typing.

Mouse test. In this test the mouse is used as a culture medium for the growth of the pneumococcus from sputum, lung substance, blood and other body fluids. In the case of sputum the specimen should be freed from surface contaminations so far as possible by washing with several changes of sterile salt solution; about 0·5 c.cm. is used for inoculation. The injections are made into the mouse's peritoneal cavity, in which pneumococci grow rapidly whilst the majority of other organisms contained in sputum, excepting *B. pneumoniæ* Friedländer and *B. influenzæ*, tend to die out. After the death of the mouse the peritoneal cavity is opened with sterile precautions and the peritoneal exudate is washed out with 4 to 5 c.cm. of sterile salt solution. If smear preparations show that the pneumococcus is present in practically pure culture in the exudate the type may be determined directly by the macroscopic agglutination test. The washings are centrifuged at a low speed to get rid of cells and fibrin; the supernatant turbid suspension is then pipetted off into a second centrifuge and spun at a high speed until the bacteria are thrown down. The supernatant fluid is discarded and the deposited bacteria are mixed with salt solution to make a suspension about equal in turbidity to a pneumococcal broth culture. This is used in a macroscopic agglutination test, being mixed in an equal quantity of 0·5 c.cm. with dilutions of each of the type sera.

When the peritoneal washings contain pneumococci in pure culture they may be tested directly after removal of the cells by preliminary slow centrifuging. The precipitin method may be used when the peritoneal washings from the mouse contain, in addition to pneumococci, an abundant growth of other organisms. The principle of the method, which was devised by Blake (1917), depends on the observation that the pneumococcus produces a soluble substance which gives a specific precipitin reaction with homologous antipneumococcus serum; the other organisms present do not produce any substance which interferes with the reaction.

Culture test. An 18-hour-old broth culture is centrifuged and the pneumococci are resuspended in salt solution and tested by the macroscopic agglutination method as in the case of the pneumococci obtained from the mouse's peritoneal washings. The immunological distinction between the types is remarkably sharp, provided virulent actively growing cultures are employed, and the simplest tests are sufficient for their differentiation. For instance, well-grown colonies on a blood-agar plate may be tested by mixing a little of the growth in tiny drops of undiluted immune sera on a slide and examining under a low power of the microscope. In heterologous sera the pneumococci form fine emulsions without a trace of agglutination, while in the homologous type serum clumps are formed immediately the cocci come into contact with the serum (author's observations).

Test of urine of patient. It has been shown that the pneumococcus liberates both in culture and in the animal body a soluble substance which frequently, and especially in severe cases, appears in the urine of a person suffering from pneumonia. Its presence may be demonstrated by means of the precipitin reaction which is specific for the type of pneumococcus causing the disease.

Specific Soluble Substances.

Investigation of pneumococcal soluble substance in relation to type-specificity has become of great importance in bacteriology, as it has provided the first instance in which serological distinctions of type have been shown to depend on differences which can be detected by ordinary chemical methods. Dochez and Avery (1917) showed that in young broth cultures from which the pneumococci had been removed by filtration, there was present in solution a substance which yielded a precipitate on the addition of homologous antipneumococcus serum. As this substance was found in the broth during the first 12 hours of growth, i.e. before death and autolysis of pneumococci had occurred, they were of the opinion that it was a product of the bacterial cell, and that it was liberated as a result of the cell's vital activities.

Each of the three principal types of pneumococci, I, II and III, have been shown to produce a specific soluble substance which reacts only with the antiserum prepared against the same type. The chemical characteristics of the three type-specific polysaccharides is considered on p. 173.

Solutions of the pneumococcal carbohydrates obtained in purified form by Heidelberger and Avery cause precipitation in high dilutions when mixed with antisera prepared with intact pneumococci of the corresponding types. The limits of delicacy of the specific precipitation with the substances obtained on different occasions range in the case of Type I from 1 in 4 million to 1 in 8 million, Type II from 1 in 2 million to 1 in 6 million and Type III from 1 in 5 million to 1 in 6 million. The reactions are highly specific. Type I carbohydrate will not precipitate Type II or

Type III antisera, and the precipitating action of Types II and III carbohydrates are similarly confined to the corresponding antisera.

Injection of the purified carbohydrates into rabbits does not provoke any antibody response, i.e. they are apparently incapable of acting as antigens, although they react specifically with antisera prepared with intact pneumococci. That such precipitation is due to the union of the carbohydrate with the antibody contained in the serum is confirmed by the fact that the carbohydrate can be extracted from the precipitate with its specific reactive properties unimpaired. Avery and Heidelberger conclude that the carbohydrate in the intact bacterial cell must be combined with some other substance which confers on it the property of acting as an antigen. This carbohydrate complex is the essential antigen which determines the serological type of the pneumococcus.

Protein Constituents of the Pneumococcus.

A second constituent of the pneumococcal cell, viz. the bacterial protein, has also been studied by Avery and Heidelberger (1923, 1925). This substance, which probably comprises a mixture of proteins, is referred to as bacterial nucleoprotein. It is antigenic and gives rise to an immune serum which reacts with pneumococcus protein irrespective of the type from which it is derived. Thus an antiserum prepared with the nucleoprotein of Type I is precipitated equally well by protein solutions obtained from all three types. On the other hand, antiprotein sera do not agglutinate type-specific pneumococci, nor do they give a precipitate with solutions of the pneumococcal carbohydrates. Such sera are species-specific but not type-specific. According to Gaspari, Sugg, Fleming and Neill (1928) the production of species-specific antibodies may be decreased by heating the pneumococcus vaccine at temperatures between 55 and 100° C. without any comparable effect upon the production of type-specific antibodies.

Type and Species Specificity.

The dominant characteristic of an immune serum prepared with intact and virulent pneumococci is the property of agglutinating the homologous type of pneumococcus and precipitating with a solution of the specific carbohydrate derived from it. In the production of type-specific antibodies reacting with the carbohydrates, the morphological integrity of the pneumococcus cell is an essential factor. If the bacterial cell is disintegrated by freezing and thawing and the remnants of the formed elements removed by filtration, the clear solution, although it contains the two important cell constituents, viz. the protein and the carbohydrate, is incapable of inciting the formation of these antibodies. The antiserum which it produces is identical with the antiprotein serum referred to above. It is completely lacking in type-specificity, and the antibodies contained in it react indifferently with protein solutions from all other types of pneumococci and even to a slight extent with proteins

of related cocci, e.g. *S. viridans* (Lancefield, 1925). Thus only one of the two constituents—the protein—contained in the solutions of dissolved pneumococci is antigenic. The second—the specific soluble substance—is as ineffective antigenically as it is in the form of the purified carbohydrate. This latter substance, nevertheless, endows the pneumococcus with its type-specific characters, and if, as in the change from the smooth virulent form to the rough avirulent (see p. 211), the pneumococcus loses its capacity to produce soluble substance, it can no longer be identified with its original type.

As stated above, Avery and Heidelberger assume that the specific carbohydrate is combined in the intact cell with some other substance which confers upon it the property of evoking in the rabbit the formation of antibodies which react with the free carbohydrate. On dissolution of the cell the combination is destroyed and the carbohydrate is deprived of its power of functioning as an antigen. Since it is believed that antigenic action is only possible with bodies of a protein nature, it is justifiable to infer that the carbohydrate is united with pneumococcus protein, though it has not been determined whether this is identical in chemical structure with the bacterial nucleoprotein obtained as the result of the analytical methods referred to above.

It will be noted that the soluble carbohydrate of the pneumococcus may be classed as a bacterial haptene. Haptene is the term employed by Landsteiner (1919) to denote chemical components which have the property of reacting specifically with antibodies. When they are united with bacterial protein, haptenes are fully antigenic, i.e. they can stimulate the production of antibodies *in vivo*. But they do not possess this latter antigenic capacity when they are dissociated from protein.

An antipneumococcus serum, as ordinarily prepared, generally contains, in addition to the sharply defined type-specific antibodies, others which react with the common protein of the pneumococcus. The latter are present in more or less abundance according to the care taken in maintaining the morphological integrity of the cell before injection into the animal body. In the case of an organism so susceptible to autolytic changes as the pneumococcus, it is manifestly impossible to obtain suspensions completely free from disintegrated cell-constituents in solution. Even if this ideal were possible of attainment one might anticipate some dissolution of the bacterial cell before it reached the seat of antibody production in the animal body.

The presence of these protein antibodies in a pneumococcus serum gives rise to cross-reactions with heterologous types. Such reactions, though only slight with sera prepared with actively growing virulent cultures which have been killed by heat before autolysis has set in, may cause the greatest confusion in serological analysis when the above conditions are not observed. Moreover, as already mentioned, avirulent cultures in the R form do not manufacture soluble substance and no longer possess the type-specific complex. As a result, sera produced by such strains resemble

antiprotein sera and react with the free protein in pneumococcal suspensions irrespective of type.

The character of the precipitate formed differentiates the type-specific reaction from that which results from the action of the protein antibodies. Type-specific serum forms with clear solutions of the soluble carbohydrate a hyaline mass which is difficult to break up. In the agglutination reaction with whole virulent bacteria the precipitation following the union of the antibody with the soluble carbohydrate occurs on the periphery of the bacteria, and their bodies are bound together into firm masses. Antiprotein sera do not form a precipitate with the soluble substance, and the deposit in an agglutination reaction as well as in a precipitin test is loose and readily shaken up to a finely granular suspension.

Avirulent or R cultures (see p. 209) produce sera which, like the antiprotein sera, form with pneumococcal suspensions finely granular deposits which are readily resuspended. These anti-R sera contain no antibodies to react with the soluble substance, and when they are mixed with autolysed virulent suspensions the deposit formed is the same in character as if the suspension had been formed of R pneumococci alone. Although in such an autolysed suspension the appropriate type-specific serum would give a type reaction, there would be no indication of type revealed by the anti-R sera.

It is not difficult to realize the importance in serological differentiation of pneumococci of employing for the preparation of sera cultures which have not only maintained their virulence but also their morphological integrity. These two factors are equally essential in the suspensions used for the determination of type. Degeneration in virulence is attended by loss of the property of forming soluble substance, and dissolution of the cell leads to dissociation of the type-specific complex. In each case the result is failure to bring out the sharp definition characteristic of the pneumococcal types.

This well-marked type specificity is clearly due to the presence of the soluble carbohydrate, which, as has been shown, is different in structure for each of the three chief types of pneumococci. In addition to the latter there are an indefinite number of types comprising Group IV, and each of these appears to be equally well-defined in its immunological reactions. Although it has not been proved by chemical analysis, it is probable that a different variety of carbohydrate enters into the composition of each type and is responsible for its specificity. That the supply of specificity-determining carbohydrates will not be readily exhausted can be seen from the fact, pointed out by Pryde, that the number of possible carbohydrates obtainable from 12 molecules of glucose is somewhere in the order of 10^{18}.

Persistence of Type Characters.

The remarkably clear definition of the pneumococcal types is paralleled by the persistence with which the type characters are preserved in

artificial cultivation under suitable conditions. After many years sub-cultivation the chief type strains show no divergence from their original characters, provided that their virulence is maintained by occasional passage through animals. Whether pneumococci may change from one type to another under natural conditions, e.g. in human infections, has not been shown, though it is difficult to conceive that the chief types, as well as the innumerable serological varieties of Group IV, are absolutely fixed and unalterable. It is well known that after recovery from pneumonia the infecting type, I or II, tends to disappear from the respiratory tract and is often replaced by a Group IV strain. Frequently several different varieties of Group IV may be found in the sputum of a convalescent pneumonia patient. According to the more generally accepted view, the chief types die out and the Group IV strains, originally present in the nasopharynx before the development of pneumonia, can alone be demonstrated. As an alternative to the above hypothesis one may consider the possibility, as the author has done (Griffith, 1928), that under the influence of the immune substances developed during recovery the pneumococcus responsible for the pneumonia may assume different serological characters, that is to say, the Group IV strains may be derived from one or other of the chief types.

Modification Experiments.

Morgenroth, Schnitzer and Berger (1925) have reported that they were able by special methods to transform pneumococci into streptococci; Reimann (1927), following the methods devised by Morgenroth and his collaborators, repeated their experiments and also obtained variants. These he identified with the R form of pneumococci obtained by various other methods. The R pneumococcus produced on blood-agar colonies resembling those of *S. viridans*, but, unlike the latter, the R colonies were invariably bile-soluble though slightly more resistant to the action of bile than the type-specific pneumococcus.

The writer (Griffith, 1928) has described a method by which one type of pneumococcus can apparently be converted into another. The principle of the method is to supply to the living R form of one type of pneumococcus a pabulum consisting of a dense suspension, killed by heat, of a virulent pneumococcus of another type from which to build up its type-specific antigen. The change of type has been obtained only when the mixture of living and heated cultures are injected into mice, and not in the test-tube. A preliminary observation which suggested the procedure was the discovery that the attenuated R form of the pneumococcus when inoculated subcutaneously into a mouse together with a mass of killed virulent culture of the same type readily reverted to the original type-specific form. The R form inoculated alone rarely reverted unless very large doses were given, e.g. the deposit of 50 to 100 c.cm. of broth culture. The temperature to which the virulent culture was heated exercised an important influence on the results and conversion from one type into

another was rarely obtained with suspensions heated higher than 60° C., though in the case of Type II steamed cultures were effective in causing the corresponding R form to become virulent. On the other hand, Type I virulent suspension which after heating to 60° C. caused the R form of Type I to revert to the S form lost this property when killed at 100° C. Thus the substance in the virulent Type I culture which was utilized by the R pneumococcus to build up its type-specific form was apparently destroyed by heat at a lower temperature than the Type II substance.

The following example gives the details of an experiment in which a rough Type II strain was changed into a virulent Type I. A virulent Type I culture grown in glucose broth was centrifuged and a thick suspension of the deposit was heated in a sealed tube at 60° C. for 15 minutes. Four mice were injected subcutaneously, each with the deposit of heated culture alone, and were killed 10 days later. The tissue from the seat of injection was removed and emulsified. Plate cultures made from the emulsion remained sterile and mice injected with it into the peritoneal cavity survived. It seemed clear that no living Type I pneumococci remained alive in the culture heated to 60° C. for 15 minutes. In the experiment proper two mice were inoculated subcutaneously, each with the heated deposit of Type I (half the amount used for the controls) together with 0·25 c.cm. of a living blood broth culture of the R form of Type II. Both mice died, in 3 and 5 days respectively, of pneumococcus septicæmia and virulent S cultures of Type I were grown from the blood.

In a similar manner a rough avirulent Type I strain injected into mice together with heated virulent culture of Type II was changed into a virulent S II pneumococcus, and an R form of Group IV acquired the characteristics of a virulent Type I or Type II according to the type of heated S culture injected along with it. The typical mucinous colonies of Type III were obtained from mice which were inoculated with living strains of R I or R II together with the S culture of Type III killed by heating to 60° C.

Neufeld and Levinthal (1928), using the method described by Griffith, also obtained transformation of pneumococcus type. Mice inoculated with an avirulent R strain of Type I together with heated suspension yielded virulent S culture of Type I, when the accompanying killed suspension was Type I, and virulent S culture of Type II, when the killed suspension was Type II.

Virulence.

Bacterial Equipment for Virulence.

Virulence in this connection signifies the capacity of the organism to multiply within the animal body, ultimately causing the death of its host. For the demonstration of pneumococcal virulence the laboratory animals most commonly employed are, in the first place, the mouse, and, secondly, the rabbit. The latter is rather less susceptible than the mouse, and, though the effects of the same strain generally correspond in the two animals, it has been observed that certain strains of Type III exhibit a

much lower virulence for the rabbit than for the mouse. Pneumococci contained in human lesions, as well as cultures from such lesions freshly isolated on suitable media, invariably possess sufficiently developed virulence to cause a fatal result on inoculation into the mouse.

Pneumococcal virulence has not been shown to be related to any one function of the bacterium, such as the formation of a toxin, but depends apparently on the mechanism of its growth in the animal body with which is associated the formation of the capsule. There are, however, in the structure of the pneumococcus and in its products certain features which are associated with virulence, and are not exhibited by avirulent strains. These features make up the bacterial equipment for virulence.

A typical virulent pneumococcus produces on blood-serum agar a translucent colony which is characterized by a smooth shiny surface and almost fluid or only slightly sticky consistency ; the latter varies with different batches of blood-media and with different serological types. Such is the normal appearance of a colony grown from human lesions or from the blood of an infected animal. Where cultures are exposed to influences, to be described later, which result in loss of virulence, the attenuated pneumococcus forms a more opaque colony with a dull or finely granular surface when viewed by reflected light ; the colony breaks up on being touched with the point of a spatula or may even move as a whole on the medium. From the appearance of the surfaces these colonies are known as the smooth, or S form, and the rough, or R form, the former being virulent and the latter attenuated (Griffith, 1923 ; Reimann, 1925 ; Amoss, 1925). Both varieties of colony produce a uniform growth in broth, but the R form may exhibit a very fine granularity with consequently a slightly greater tendency than the S form to deposit. This is no doubt due to a tendency of the R cocci to form clumps, and it is this clump formation which probably causes the R form to appear less readily soluble in bile than the S form.

The high virulence of the S pneumococcus is associated with vigorous capsular development, and there is reason to believe that the capsular substance is composed of the same carbohydrate material as the type-specific soluble substance, since loss of capsule is associated with loss of soluble substance. It has been suggested that the latter is retained about the pneumococcus as a protecting and insulating layer thus forming the capsule. In addition, large amounts of soluble substance are formed during the course of a pneumococcus infection, and are found in all the body fluids. Since the substance possesses the power of neutralizing pneumococcus antibodies, these antibodies are diverted from the living structure of the bacterium. The R form of the pneumococcus which ordinarily has no capsule and does not produce soluble substance is readily taken up by phagocytes and has little opportunity to multiply when inoculated into the body of the mouse. There is thus a strong indication that the capsule is largely made up of the type-specific soluble polysaccharide, and is part of the defence mechanism of the bacterial cell.

The necessity of employing cultures of high virulence in immunological work on the pneumococcus was stressed by Neufeld, and recent work on the association of virulence with type characters has emphasized its importance. Pneumococci are prone to lose their virulence in artificial culture, especially on solid media, and it has been shown that this is the consequence of changing from the S to the R form. Retention of virulence has come to mean, to a large extent, prevention of this change. While it is true that passage through a mouse will eliminate the R form from a partially attenuated culture, such attenuation should none the less be avoided if a state of constant virulence is aimed at. One cannot be certain that the S form from such a culture may not have suffered some damage to its virulence-equipment which can only be repaired by numerous animal passages.

The S to R change does not occur in pneumococci which remain alive but do not multiply, and it is on this principle that successful methods of preserving virulence are based. There is the method first published by Heim and followed independently by Neufeld, which consists in the rapid drying of bacteria in albuminous fluids. Thick layers of blood containing pneumococci or the spleen of a mouse which has died of pneumococcal septicæmia are rapidly dried in a desiccator and enclosed in sealed tubes. Provided that the tissues were rich in pneumococci to begin with, it is possible to keep strains viable and fully virulent for six months ; cultures have been recovered after 2 to 3 years and even longer (writer's observation). For stock strains in constant use the following method is reliable ; it depends on preservation in the cold. A culture is made from the heart-blood of a septicæmic mouse in broth containing 50 per cent. defibrinated rabbit-blood. After incubation overnight the culture is stored in the refrigerator at a temperature of about 4° C., a test of purity being made at the same time. Under these conditions it will remain alive without altering in virulence for 4 to 6 weeks, and subcultures may be made from it as required.

The weakening of virulence which the pneumococcus is liable to undergo in cultivation outside the animal body may be accentuated by various means :

1. Successive cultures of a virulent pneumococcus in immune serum cause a gradual attenuation and may lead finally to a complete loss of virulence. The influence of the serum is specific in character, and is almost exclusively confined to strains of homologous type ; undiluted serum with a high content of antibodies is most effective. The changes which take place in the original strain may be studied by plating on fresh blood-agar the different generations of serum cultures. The first generation, where growth takes place in the form of a firm gelatinous mass, yields on plating a mixture of R and S colonies, the latter predominating. The proportion of R colonies increases in subsequent generations, and with complete attenuation of the whole culture the S colonies disappear. In addition the character of the growth changes, the pneumococci being

diffused uniformly throughout the serum. R colony cultures obtained at any stage of the passage through serum are incapable of multiplying in the mouse, and do not produce a fatal septicæmia (unless reversion to the S form takes place), though large doses intraperitoneally may cause death from toxic action. There is some evidence, as shown by differences in capacity to revert to the virulent form, that strains from individual R colonies are not equally attenuated.

2. Growth in bile causes attenuation and changes in the character of the colonies similar to those obtained in immune serum.

3. Other methods of producing attenuation are by growth in optochin, in meat infusion, in acid broth and at temperatures over 39° C.

4. Attenuated R strains have also been obtained from the blood of highly immunized horses inoculated with living virulent pneumococci.

Pneumococcus cultures which have become partially attenuated in the course of cultivation on artificial media and have dissociated into a mixture of R and S forms regain their virulence after passage through animals. The effect is in part the result of the elimination of the attenuated forms, and the survival of those best suited for multiplication in the animal body.

Selection of particular cells, however, is not the only mechanism involved in increase of virulence. Avirulent strains derived from single pneumococci, and wholly composed of R forms, may revert to the virulent S form after inoculation into animals (see p. 209). It must also be mentioned that Dawson and Avery (1927) and Dawson (1928) found that virulence and type-specificity could be restored to R pneumococci by growth in an antiserum prepared by immunizing rabbits with avirulent R pneumococci. Moreover, Felton and Dougherty (1924) have reported that virulence may be increased *in vitro* by frequent transfers in milk at 4-hourly intervals ; they also found that peptone in 2 per cent. solution maintained and even increased the virulence of a strain of pneumococcus. The possibility of determining virulence by the supply to the growing organism of definite chemical substances is a subject worthy of further study.

Pneumococci obtained from the lesions of pneumonia patients are generally of high virulence for mice, causing fatal septicæmia within 48 hours in a dose of 10^{-7} c.cm. of broth culture. On the other hand, these same sources have yielded cultures which failed to kill in much larger doses, and Neufeld and Händel found strains of pneumococci which killed mice when injected in very small doses, but only after a period of 5 to 7 days. As a rule pneumococci freshly isolated from human beings only attain their maximum virulence for a particular species after several passages through animals of that species. Moreover, high virulence for one species does not necessarily signify equally high virulence for another species. Tillet (1927) examined 11 strains of Type III, obtained from cases of pneumonia, 10 of which were of low virulence and 1 of high virulence for rabbits, while all killed mice in a dose of 10^{-7} c.cm.

Strains of Group IV from the normal mouth often exhibit low virulence for mice, though some may be as highly virulent as those from human lesions. While virulence of pneumococci for animals cannot be taken as a measure of their virulence for man, there is some evidence that Group IV strains produce a less severe form of pneumonia in human beings than, for example, Types II and III (see p. 203).

Poisonous substances causing acute symptoms and death in animals have been extracted from the bodies of pneumococci by various methods, e.g. by grinding in a frozen condition, by autolytic dissolution, by the action of bile-salts and by alternate freezing and thawing. Some of these substances have been shown to be preformed in the bodies of the bacteria, while others are apparently split-products of the bacterial protein, but it is not conclusively proved that these are specific pneumococcal toxins.

It has been suggested that in lobar pneumonia intracellular toxic substances are liberated from the pneumococci after their death and lysis, and, finding their way into the circulation, set up the general symptoms of acute fever and toxæmia. This hypothesis is not in accord with the course of the disease, which sets in abruptly and in which high fever and general symptoms occur soon after the onset. The facts seem to indicate rather that the pneumococcus does, in fact, sècrete a diffusible toxin in the human body. While it is certain that the pneumococcus does not produce *in vitro* any substance to rank with diphtheria or tetanus toxin, recent experience with the scarlatinal streptococci shows that it would not be safe to exclude the presence of a soluble toxin in filtrates of broth culture on the grounds of negative results in animals. That pneumococci do form a specific substance which diffuses throughout the medium in broth cultures is well known. This substance, which is present in very young and actively growing cultures, is actually secreted from the bodies of the pneumococci, and is not a product of the disintegrated cell. It can be demonstrated occasionally in the blood of human beings suffering from pneumonia and is frequently present in the urine. Although in fluid cultures it has not been shown to possess a high degree of toxicity for animals, there is a possibility that in the nascent condition in the human body it may be more effective, and may be responsible for the symptoms of intoxication in lobar pneumonia. The amount of the substance excreted in the urine varies with the intensity of the pneumonic condition and a progressive daily increase is generally of grave significance. Dochez and Avery (1917) have shown that the mortality in lobar pneumonia is much lower when the urine does not contain the specific soluble substance.

Antigenic Characters in Relation to Virulence.

Agglutinins. It has been shown on p. 207 that pneumococcus agglutinins are of two varieties, one of which reacts with type-specific (virulent) pneumococci, and the other with the R (avirulent) form.

Absorption with R pneumococci of a serum containing both varieties of agglutinin removes the anti-R agglutinin and leaves the type-specific agglutinins undiminished. It is possible also (Reimann, 1926) by absorption with type-specific pneumococci to remove from a serum all the type-specific agglutinin, leaving the anti-R agglutinin. The type-specific agglutinins are remarkably stable and persist in immune sera kept at room temperature for many years though with gradually diminishing titre.

Precipitins. The reactions of precipitation and agglutination with pneumococci exhibit so close a correspondence that it seems certain that they must be due to the interaction of identical substances. Each phenomenon is provoked by the same antibody, which forms a precipitate with the appropriate antigen in solution, and causes an agglutination of the antigen in a particulate state, whether as whole cells or as finely divided protein.

Bacteriotropins. A definite advance towards the elucidation of the action of serum from immunized animals upon pneumococci was made by Mennes (1897), who showed that leucocytes from normal animals as well as those from immune animals were unable to ingest virulent pneumococci in the presence of normal serum. The substitution of immune serum on the other hand caused an active phagocytosis. Neufeld and Rimpau (1904) showed that the influence of the immune serum, which they termed bacteriotropic action, in causing phagocytosis was exerted upon the organisms and not upon the leucocytes. They first incubated leucocytes for 20 minutes at 37° C. together with antipneumococcus serum, and after removal of the serum by centrifuging added the leucocytes to pneumococci, with the result that no phagocytosis occurred. When pneumococci were similarly treated with the serum, and after careful washing were brought into contact with untreated leucocytes and normal serum active phagocytosis occurred. In this bacteriotropic action of immune serum the complement played no part, i.e. serum heated at 56° C. was still active. Protection with immune serum runs parallel with bacteriotropic action, both in the test-tube and in the animal body. In both cases also the effect of the serum is limited to pneumococci of the same type as that with which the serum was produced.

It is probable that these antibodies in the serum, termed bacteriotropins, act by neutralizing some substance on the surface of the pneumococcus which prevents the leucocytes from ingesting them. This substance is no doubt associated with the capsular material and the specific soluble carbohydrate. Sia (1926) has shown that the addition of a small quantity of soluble substance of homologous type enabled even a small number of avirulent pneumococci to grow in the serum and leucocytes of animals which possess the power to destroy ordinarily such pneumococci in relatively large numbers. The mechanism of the reaction is not understood, but it does not appear that the soluble substance injures the leucocytes.

The attenuating influence of immune serum *in vitro* upon pneumococci may be attributed to the bacteriotropic substances. In this case the descendants of the virulent pneumococci are deprived of their power of producing capsules and soluble substance, and when injected into animals in this condition are readily taken up by normal leucocytes. To what extent this process of degradation occurs in the natural recovery of an animal from a pneumococcus infection is not known. Reimann (1927) has shown that R forms can be produced *in vivo* under suitable conditions, but obtained no proof that this change was an essential preliminary to the destruction of the S forms. The latter might disappear entirely without any R forms having been observed. It is clear, however, that the influence of these bacteriotropic substances may serve a useful purpose in acting as a check on the multiplication of the virulent pneumococci, since there is this tendency for their descendants to be attenuated in immune serum. In a number of instances, Wadsworth and Sickles (1927) have recovered avirulent strains from horses undergoing immunization with living pneumococcus cultures.

Opsonins appear in certain cases to play a part in natural immunity against the pneumococcus. Ungermann (1911) studied a pneumococcus strain which was virulent for mice but not for rabbits. He found that normal rabbit-serum had an opsonizing action, whereas normal mouse-serum had no such effect. Perhaps the experiments of Sia referred to above may be explained on the assumption that the soluble substance added to the serum-leucocyte mixtures neutralized the normal opsonins.

Tillett (1927), however, has shown that some other factor than phagocytic action is operative in the natural immunity of rabbits against many Type III strains. These strains, which were obtained from pneumonia patients, were capsulated and highly virulent for mice. Inoculated intravenously in rabbits they caused a fluctuating bacteriæmia, the blood finally becoming sterile and the rabbits surviving. Yet when these S forms of the Type III pneumococci, avirulent for rabbits, were mixed with normal rabbit-blood no phagocytosis was observed though the R forms were rapidly taken up.

Specific Therapy.

Production of Antisera.

The observations of Fornet and Müller (1908) on antiserum production in animals have supplied the basis of the methods in general use at the present day. The principle is the daily injection for 3 or more successive days followed by a rest of 7 days, this procedure being repeated until an active serum is produced. Cole and Moore (1917) who were the first to apply this method to the preparation of antipneumococcus serum, give a description of their technique for the immunization of horses. A similar technique is applicable to rabbits, and, according to Cole and Moore, the doses should be small, since it has been found that very large doses inhibit

the immunity response. Individual rabbits, however, vary greatly in this respect. The writer has obtained sera of high titre, both agglutinating and protective, after 7 series of injections of killed culture in a total period of 6 weeks. The doses were large, ranging from 8 c.cm. to the deposit of 30 c.cm. of broth culture, and were given sometimes on 2, at others on 3 successive days, with periods of rest varying from 5 to 7 days.

The necessity of using virulent strains for preparing pneumococcus antisera has been stressed in earlier sections, and there is no doubt that the character of the strain is an important factor in successful immunization. The criterion of high virulence for the mouse does not, in the case of Type III pneumococcus, necessarily indicate suitability for use in rabbits. The majority of Type III strains, though highly virulent for the mouse, are of low pathogenicity for rabbits, and this fact has, no doubt, much to do with the difficulty of inciting the production of type-specific antibodies in the latter animals.

Whether there is any advantage in continuing immunization with living pneumococci is by no means certain. There is no doubt that very potent sera can be produced by the injection of killed culture alone, provided the strain was originally of high virulence. Living attenuated culture is useless for the preparation of protective serum, and, as is now known, this is due to the absence of type-specific antigen in such strains. This essential antigenic complex is liable to disintegration in living cultures, both prior to and subsequent to inoculation, with the result that non-type-specific antibodies are produced in the serum. Moreover, animals, even though hyperimmunized, are prone to develop endocarditis and die.

Most writers agree that the intravenous method of injection is the best for the production of high-titred antipneumococcus serum. The formation of protective substances in the serum can be induced by subcutaneous injection, but the sera never attain a sufficiently high titre even after months of treatment of the animals.

Standardization of Antipneumococcus Serum.

The potency of antipneumococcus sera for therapeutic use is determined by protection tests on mice. There are two chief difficulties in the way of obtaining a method as exact as, for example, that employed in the case of diphtheria and tetanus antitoxic sera. In the first place it is impossible to establish a minimal lethal dose, since pneumococci are very variable in their virulence, and with some highly virulent strains even a single organism is capable of causing a fatal infection. Secondly, the law of multiple proportions, that is to say, the constant relationship between the size of infecting dose and the amount of serum necessary to protect, is valid only within very narrow limits. The substitution of a relative standard such as the estimation of bacteriotropic power and agglutination has not so far been successful. Agglutination at least, does not appear to bear a constant relation to protective power, for sera have been prepared which give good protection but do not agglutinate.

While the protection tests at present available do not admit of exact estimation of the protective antibodies in a serum, they provide a practical method of establishing a standard of potency below which a therapeutic serum should not fall. There is, as the American observers have pointed out, a well-defined limit, which is constant and differs for the different types of pneumococci, to the strength of a serum which can be obtained by present methods of immunization. If the latter can be improved in the future it will be necessary to aim at more exact methods of titration.

The American workers on the basis of their own experimental work, and of the previous work of Neufeld have fixed a definite standard of strength to which sera used for therapeutic purposes should conform. A serum of minimal potency must protect a mouse against at least 0·1 c.cm. of a pneumococcal culture which in a dose of 10^{-7} will cause fatal septicæmia in a mouse of 20 gm. within 48 hours. In the actual test mice weighing 18 to 22 gm. are used, and the largest amount of an 18-hour broth culture of the homologous pneumococcus against which a constant quantity of serum, viz. 0·2 c.cm. will protect is determined. For convenience one amount of serum is used, though theoretically it would be better to titrate several amounts of serum against varying doses of culture. The serum is diluted with broth so that the fixed amount of 0·2 c.cm. is contained in a total bulk of 0·5 c.cm. Dilutions of broth culture are prepared so that each dose, ranging from 0·2 to 10^{-8} c.cm., is contained in 0·5 c.cm., the dilutions being made with the same broth as that in which the bacteria are grown. A separate pipette is used for making each dilution, and the interval between their preparation and injection should be as short as possible. The serum and culture dilutions are mixed before injection into the peritoneal cavity of the mouse. First the required amount of serum is drawn into a 2 c.cm. syringe and then the culture dilution ; if the series of injections is begun with the smallest dose of culture the same syringe may be used throughout.

A standard antipneumococcus serum should always be tested at the same time against similar doses of culture. The virulence of the culture is tested on three mice which are inoculated with doses ranging from 10^{-6} to 10^{-8} c.cm., and should develop fatal septicæmia within 48 hours. For the test mice the period of observation may be fixed at 5 days.

Slight modifications of the technique are permissible so long as a fixed plan is adhered to. For example, the serum may be injected alone 3 hours or even 24 hours before the culture, as recommended by Neufeld ; the period of observation of the test mice may be shortened to 3 or 4 days. There is one essential precaution which cannot be neglected, namely, that the test culture should be of high virulence and should invariably kill at the level fixed. A standard serum should never be omitted, since a comparison with previous results will reveal any variation in the virulence of the test culture and a double series of mice should be used for each serum under examination. When the above-mentioned technical considerations are fulfilled it has been shown by comparative tests undertaken in different

laboratories (in America, Denmark and England under the auspices of the Health Committee of the League of Nations), that several samples of therapeutic serum can be placed in the same order as regards protective potency.

Serum Therapy.

Human lobar pneumonia. Since pneumococcus antisera are strictly type-specific, the type of pneumococcus causing the infection must be determined by one or other of the methods described before serum treatment can be attempted. A serum prepared with one type has no more influence than normal horse-serum on the course of an attack of pneumonia due to another type, and the production of an efficacious polyvalent serum is at present a somewhat remote possibility, although the antibodies from monovalent sera may be concentrated and combined in a single preparation. Huntoon (1921) absorbed the antibody from antipneumococcus serum with homologous living pneumococci, and recovered it from the bodies of the cocci by washing with weak alkaline solution. Felton (1925) precipitated the antibody from antipneumococcus serum, freed from 90 per cent. of serum protein, by 20-fold dilution with water or weak acid solution, and redissolved it in salt solution.

Extensive trial of antipneumococcus serum as a therapeutic agent in human infections has been made only in the case of Type I serum, as the difficulties experienced in preparing antisera in horses from the other types of pneumococci with a sufficiently high protective value have not been overcome. Quite recently, however, R. L. Cecil (1928) has reported promising results obtained with a polyvalent serum preparation, concentrated by Felton's method, in the treatment of Types I and II pneumonia.

When one recalls that in protection experiments on mice no increase in the amount of serum administered will save the life of a mouse if the dose of pneumococci exceeds a certain size, and that a sufficient concentration of antibodies in the tissues of the animal is necessary to prevent multiplication of the injected organisms, the rationale of two essential recommendations for successful treatment becomes plain. In the first place the serum must be given at the earliest possible stage in the disease in order to counteract the multiplication of the pneumococci and the saturation of the body fluids with their products, one of which—the soluble substance—is able to neutralize the specific protective substances. Secondly, the serum must be injected repeatedly and in large doses into the blood-stream to maintain the necessary concentration of the specific antibodies. A good description of the method of administration of serum and of the precautions necessary to avoid anaphylactic shock is given by Avery, Chickering, Cole and Dochez (1917).

The injection of a sufficiently large dose of homologous antipneumococcus serum early in a case of Type I pneumonia usually causes a marked improvement in the general condition of the patient with relief from toxæmia. The local lesion in the lungs ceases to extend, and the blood

when pneumococci were previously found, becomes sterile. There is no change in the rate of resolution of the lung tissue already involved, this process appearing to be independent of immunity reactions.

While there is no doubt that these favourable effects are in many cases directly attributable to the specific therapy, clinical considerations alone are an insufficient guide to the value of the serum. Changes equally sudden and striking are characteristic of spontaneous recovery in pneumonia. The best single criterion of the value of the serum is without doubt its effect on bacteriæmia. Bloomfield (1923) found from an analysis of a small number of cases that patients with a negative blood culture when the serum was administered invariably recovered, irrespective of the stage of the disease. Bacteriæmia late in the disease is of grave import, and is but little influenced by serotherapy, though Bloomfield thought that certain individual cases in his series were saved by the serum. The influence of bacteriæmia on the prognosis in lobar pneumonia has been emphasized by other observers. Avery and his associates (1917) reported a mortality of 55·8 per cent. in a series of 136 cases with positive blood cultures compared with 8·3 per cent. mortality in 312 cases in which the blood cultures were negative. Baldwin and Rhoades (1925) found from a study of blood cultures in 107 cases a mortality rate of 78·3 per cent. in 37 cases which yielded positive blood cultures in contrast to 10 per cent. in 70 cases with sterile blood cultures.

The final proof of the efficacy of serum treatment must be based on gross mortality statistics and the figures must necessarily be very large to provide convincing evidence. The reports so far published are conflicting and the data given insufficient. In particular, in view of the variable mortality from pneumonia, an essential control is generally wanting, viz. observations on a simultaneous group of untreated individuals of like age and condition.

In the first report from the Hospital of the Rockefeller Institute on the curative effect of serum treatment, it was stated that out of 107 cases of Type I pneumonia treated with serum, 8 died = 7·5 per cent. A subsequent report (1920) includes 88 additional cases, bringing the total to 195 treated with serum with 18 deaths = a mortality of 9·2 per cent. (This is contrasted with the fatality rate of 25 to 30 per cent. observed before serum treatment was begun.) The only grounds on which the significance of these figures can be questioned are that there was no simultaneous control group of cases (see reference to Type I mortality on p. 204). Wadsworth (1924) has reviewed the literature from 1915 to 1923 upon the serum treatment of pneumonia, and gives the results in 445 additional cases which were treated under various conditions with antipneumococcus serum of known high potency. After careful analysis of the factors liable to cause discrepancy in the results, he observes 'there would seem to be no question but that antipneumococcus serum of high potency, when promptly administered in adequate dosage, is of definite practical value in the treatment of Type I infections of pneumonia'.

Animal experiments. Cecil and Blake (1920[3]) have tested the curative value of Type I antipneumococcus serum on monkeys in which pneumonia was induced by the intratracheal injection of living Type I pneumococci. The results showed clearly that the intravenous administration of the serum freed the blood quickly and permanently from pneumococci, shortened the course of the disease and moderated its severity. The action of the serum was highly specific, normal horse-serum exerting not the slightest influence on the progress of the disease. The experiments brought out the importance of early treatment and frequent injections. But even when the treatment was started late in very severe cases, the monkey's life might be saved, though the course of the disease was not appreciably shortened.

Cecil and Steffen (1925) have made experiments on monkeys with the polyvalent (Types I, II and III pneumococci) antibody of Huntoon. Monkeys were injected intratracheally with virulent culture and treatment with antibody solution was begun 24 to 72 hours later ; each experiment included one or more untreated control monkeys. Two monkeys which received Type I culture recovered completely after the administration of the antibody solution, although before treatment both animals gave positive blood cultures, and one was almost moribund. The control monkey died on the fourth day with lobar pneumonia. The solution was also effective against Type II pneumonia, but earlier and more intensive treatment was necessary than in Type I infections. No therapeutic action was shown against Type III pneumonia and treatment was equally ineffective in the case of a Type IV infection, a result which was not unexpected, since the antibody solution was unable to protect mice against the particular strain.

While in the above instances the solution was injected intravenously, subcutaneous administration was also found to be effective in Type I pneumonia, though larger doses were necessary and the blood was less readily rendered sterile. Unfortunately there is great variability in different animals with regard to the amount of protective antibodies which appear in the blood after subcutaneous injection. The experiments made by Rhoades (1925) on the fate of pneumococcus antibodies when injected into normal animals and men, hold out little hope that the subcutaneous method of administration can be substituted for the intravenous in the treatment of human lobar pneumonia. While often a considerable degree of protective power against pneumococci, as shown by tests on mice, could be conferred on the blood, especially in rabbits, after subcutaneous injection of potent antibody solutions, in other cases no protective substances could be demonstrated even after large subcutaneous doses.

Vaccine Therapy.

Treatment of pneumonia with killed pneumococcal vaccines has been tried by Wynn (1915), Malone (1924) and others with favourable results in the early stages of the disease.

Apart from the practical issue, the possibility of successful treatment of acute pneumonia with vaccine raises interesting questions in relation to pneumococcus immunity. The period during which the symptoms are said to be substantially ameliorated and the progress of the disease checked seems too short for the elaboration of the ordinary antibodies. The possibility of the existence of a state of immunity in which these antibodies are not concerned has been frequently under consideration. The experiments on pneumonia in monkeys by Cecil and Steffen (1925) supply one instance, and a second which seems particularly pertinent is the demonstration by Hedley Wright (1927) of the rapidly enhanced clearing property of the blood of rabbits against virulent pneumococci induced by a single injection of heated culture. In regard to man, at least, it might be suggested that the vaccine incites the immediate production of antibodies by cells already sensitized through previous exposure to pneumococcus infection.

The rationale as well as the value of vaccinotherapy are well worth further experimental study, and the ease with which pneumococcus pneumonia, conforming closely to that occurring in man, can be set up experimentally in certain species of monkeys suggests that these animals might be used to check the results obtained in man.

Until definite experimental evidence in favour of vaccinotherapy has been obtained and until its efficacy has been demonstrated by observations on a large and well-controlled series of human cases of pneumonia, the indiscriminate use of various pneumococcal preparations as vaccines for therapeutic purposes is inadvisable. The varied and uncertain clinical course of pneumonia renders it difficult to estimate accurately the value of any form of specific treatment. In this connection it is sufficient to recall the difference of opinion which still exists in regard to the influence on mortality of treatment with Type I serum, although the antipneumococcal properties of the serum have been established by conclusive experimental evidence.

Prophylactic Vaccination.

Animals. Yoshioka (1922–3) and Killian (1924, 1925) have produced a high degree of immunity in white mice by vaccination with killed pneumococcal cultures. Stillman (1924[2]) showed that mice which had survived repeated exposures to a spray of living virulent pneumococci had acquired a definite resistance to subsequent intraperitoneal infection, the amount of protection obtained depending to a considerable degree on the number of previous exposures. Eguchi (1925) reported immunity after spraying with killed culture. Rabbits are readily immunized by injection of killed pneumococci and a certain degree of immunity has been found after inhalation of living pneumococci (Stillman, 1927). Experiments on vaccination of monkeys have been made in America by Cecil and Blake (1920[1 & 2]) and Cecil and Steffen (1921, 1925). Protection has been obtained against the development of experimentally induced pneumonia by

subcutaneous and intratracheal injections. It is stated that the Philippine macaque (*Macacus syrichtus*) is the most suitable species of monkey.

There is a striking unanimity, not only in the articles quoted above, but also in the literature generally with regard to the effective nature of the resistance to pneumococcal infection which can be induced even in the most susceptible animals by vaccination with pneumococci. Treatment with living pneumococci, which has obvious risks, is unnecessary, since a very high grade of immunity can be established with heat-killed cultures. The pneumococcus antigen concerned in the production of active immunity is very resistant to heat and appears not to be appreciably weakened in activity by exposure to steam at 100° C. (Tani, 1924).

Active immunity to pneumococcus is highly type-specific, though instances of a slight amount of protection have been recorded both in mice and monkeys after treatment with heterologous types (Yoshioka, 1922 ; Cecil and Blake, 1920[1]).

Virulent pneumococci of the S type are as essential for the production of a high degree of active immunity as in the preparation of a potent protective serum (Gaspari, Fleming and Neill, 1927). As a rule vaccines composed of avirulent pneumococci have not been found to increase appreciably the resistance of an animal, though exceptional instances have been noted. For example, Cecil and Blake (1920[2]), though not able to protect monkeys against experimental pneumonia by the injection of an avirulent killed culture of pneumococci, modified the course of the disease ; in one instance (1920[2]) they obtained protection through the subcutaneous inoculation of a large dose of living avirulent pneumococci. Others have observed increased resistance in mice evoked by one R culture and none by another.

For these occurrences there are at least two alternative explanations possible : (1) certain R strains, though attenuated in virulence, may retain a remnant of their S-producing equipment (Griffith, 1928) ; (2) the immunity produced may be an enhancement of the normal resistance analogous to that obtained by Tillett (1927) against a Type III strain of high virulence for rabbits by means of injection of heterologous R and S cultures. The choice between these alternative explanations must await elucidation of the nature of active immunity to pneumococcus. There is a general consensus of opinion that a high degree of acquired active immunity may be present without demonstrable antibodies in the serum. To account for this one may suppose either that antibodies affording protection are actually present, but in so small amount as to escape identification by the usual *in vitro* experimental methods, or that the cells concerned with antibody production have through the immunization been given the potentiality to secrete them rapidly on the stimulus of the infecting inoculation, or that the invading organisms are suppressed through increase of the normal capacity of the animal body to destroy pneumococci without the intervention of the known antibodies. While the evidence is in favour of a primary humoral immunity the possibility

that the resistance is in fact cellular is indicated by the local reaction of the lungs to inhaled pneumococci in animals previously exposed to this method of infection (Stillman and Branch, 1924); animals thus immunized present little if any general immunity to intraperitoneal inoculation.

That there may be a difference in the manner of production of active as opposed to passive immunity is indicated by experiments made with whole pneumococci and with dissolved pneumococci. For the preparation of potent protective sera, it is essential that the injected pneumococci should be morphologically intact. On the other hand, there is considerable evidence that various pneumococcal solutions which have been shown to be incapable of inciting the formation of type-specific antibodies may, nevertheless, give rise to active immunity (Perlzweig and Steffen, 1923; Ferry and Fisher, 1924; Perlzweig and Keefer, 1925; Meyer, 1927). In such solutions the protein-carbohydrate compound is dissociated, and the only active antigenic component is the residual protein; injection of this gives rise to antiprotein bodies which do not react with intact virulent pneumococci. These antibodies formed against the pneumococcus protein do not confer passive immunity, and if it is true that the injection of protein solutions gives rise to active immunity, some other mechanism must be postulated. It must be remembered, however, that mice, on which the majority of the immunizing experiments with filtered pneumococcal solutions have been made, acquire a high degree of immunity after injection of exceedingly small amounts of virulent culture. Even the absorption of minute quantities of dead culture through the respiratory or alimentary tract appears to promote a definite degree of resistance. It is, therefore, conceivable that the passage of a very small amount of the undissociated protein-carbohydrate molecule through the filter might suffice to elicit an active immunity response in the mouse.

Man. The ease with which active immunity can be induced in the most susceptible animals by the injection of small quantities of dead culture of pneumococci shows that vaccination of man against pneumonia is theoretically possible. The statistical evidence available is by no means conclusive as to its practical value. In 1911 and 1912, Wright (1914) made a practical trial of preventive inoculation on a large scale in South Africa, where pneumonia was very prevalent among the native mine workers. It has been found difficult to interpret the results as no attention was paid to the type differentiation of pneumococci. Lister renewed the attempt to immunize the same native population in the light of his serological classification of pneumococci occurring in South Africa. After preliminary experiments he fixed the dosage at 6 to 7 thousand million pneumococci given subcutaneously on 3 occasions at intervals of 7 days. He included in his vaccine the most prevalent types. While a definite decrease in the incidence of pneumonia and in the mortality rate was observed, he considered of still greater importance the fact that among the vaccinated workers in one mine there were no cases of pneumonia due

to the particular types contained in the vaccine. Malone (1924, 1925) in India had negative results.

Probably the most important test is that made by Cecil and Austin (1918) at Camp Upton, U.S.A. Among 12,519 vaccinated men no cases of pneumonia due to Types I, II and III occurred in those who had received two or more injections of a polyvalent vaccine containing those types. During the period of observation—10 weeks—there were 26 cases of pneumonia due to these 3 types in a control of approximately 20,000 men. A second trial on a large scale by Cecil and Vaughan (1919), in which a pneumococcus lipovaccine was used, was less good. A report (1922) on the use of lipovaccine in New York State institutions states that the results were far from satisfactory.

It is at least doubtful whether in the attempts made so far to test the value of prophylactic vaccination against pneumonia in man, the best form of antigen has always been employed. In the production of sera conferring passive immunity, considerable emphasis has been laid on the necessity of using highly virulent cultures which have been killed by heat after a relatively short period of growth before autolysis has begun. While a certain degree of active immunity in animals and apparently in man has been obtained with pneumococcal preparations which do not comply with the above conditions, it is conceivable that, as in the production of protective sera, heated saline vaccines composed of highly virulent and morphologically intact pneumococci would give the best results.

Sensitiveness to Antiseptics: Chemotherapy.

By C. H. Browning.

The pneumococcus has in general been found sensitive to the commonly used disinfectants. As an example, in experiments in which the concentrations of the antiseptic were determined which permitted or prevented growth in broth containing 5 per cent. ox-serum (previously heated for 1 hour at 60° C.), when inoculated with a small quantity of a 24-hours broth culture, and incubated for 24 hours at 37° C., Schiemann and Ishiwara (1914) obtained the following results with pneumococci:

Phenol.		Corrosive Sublimate.	
Concentration preventing Growth.	Concentration permitting Growth.	Concentration preventing Growth.	Concentration permitting Growth.
1 : 600	1 : 1,000	1 : 100,000	1 : 300,000

Simon and Wood (1914) investigated the action of a large number of dyes and concluded that the majority of basic type, especially those of the

triphenylmethane series, in a concentration of 1/100,000 in agar were inhibitory for pneumococci as tested by making a stroke culture on the medium. Acriflavine (trypaflavine) acts powerfully both on virulent and non-virulent pneumococci (Schiemann and Baumgarten, 1923). Norton and Davis (1923) found that organisms belonging to the *S. viridans* and pneumococcus groups were inhibited by dyes to the same extent, so that none could be used for the purpose of differentiating between them.

In consequence of the chemotherapeutic properties of ethylhydrocupreine (optoquine) in experimental pneumococcus infection in mice demonstrated by Morgenroth and Levy (1911), the antiseptic properties of this substance and other compounds related to quinine have been extensively investigated ; it is to be noted that freshly prepared solutions should be used (see Schnitzer, 1926). Wright along with Morgan, Colebrook and Dodgson (1912) showed that pneumococci are highly sensitive to the bactericidal action of optoquine, and that as compared with staphylococcus, for example, this action is exceedingly selective. The selective effect of optoquine has been confirmed by Neufeld and Schiemann (1913) and also by Schiemann and Ishiwara (1914), who showed that streptococci (Aronson's strain and a non-virulent culture) were about one hundred times less sensitive to optoquine when tested in broth containing 5 per cent. heated ox-serum than were virulent pneumococci, the latter organisms being killed in a concentration between 1 : 300,000 and 1 : 1,000,000. But the lethal concentration may vary somewhat according to the age and source of the organisms and individual factors. The lethal effect on pneumococci is very little diminished by the presence of human serum (Wright and co-workers) or rabbit-serum (Schiemann and Ishiwara), either when unheated or after heating at 60° C. for 1 hour ; but some variation is met with when different specimens or species of serum are examined and serum may delay the action (Solis-Cohen, Kolmer and Heist, 1917). Optoquine kills pneumococci relatively slowly, also it acts more intensely at 37° C. than at lower temperatures (Tugendreich and Russo, 1913).

Kolmer and Idzumi (1920) investigated the antiseptic action in purulent spinal fluid from pneumococcus (Type II) meningitis, and found that optoquine in a concentration of 1 : 10,000 produced sterility in from 60 to 90 minutes, but 1 : 50,000 failed in 90 minutes. Quinine hydrochloride 1 : 200 sterilized the fluid in 45 to 60 minutes, but a concentration of 1 : 400 failed to sterilize in 90 minutes. In thick pus from the pleura of a human case, Type I pneumococci were killed *in vitro* at 37° C. within 30 minutes by the addition of an equal volume of 1 : 1,000 solution of optoquine in saline, whereas half this concentration of the drug failed to kill the organism in two hours (Kolmer and Sands, 1921). Schnabel (1920) states that dilutions of optoquine of the order of one in a million or higher may be detected by the effect of the drug in interfering with the reduction of a solution of methylene blue when added to a fluid culture of pneumococci.

The investigation of substances chemically related to optoquine, including analogous cupreine-derivatives, quinine and other members of the quinine group of alkaloids, has shown that *in vitro* pneumococci are in general specially sensitive to optoquine as compared with the other compounds (Morgenroth and Levy, 1911 ; Tugendreich and Russo, 1913 ; Morgenroth and Bumke, 1914, 1918 ; also Morgenroth and Schnitzer, 1924, in contradiction of Felton and Dougherty, 1922 ; see further Kolmer and his co-workers, 1917). Iso-propylhydrocupreine alone approaches optoquine in its action (Morgenroth and Bumke, 1914), although individual strains may be peculiar in their susceptibility, e.g. to vuzine (Morgenroth, 1919). Of all the substances investigated, including other cinchona compounds and synthetic derivatives, optoquine would also appear to be the most effective *in vivo* on generalized infections so far as determined by experiments on mice (Felton and Dougherty, 1922).

Considerations regarding the events occurring in the exudate in pneumonia at the stage of resolution led Lamar (1911) to investigate the action of soaps on pneumococci. He found that sodium oleate in suitable concentrations (1 to 0·5 per cent. added to a broth culture) both killed and dissolved pneumococci quickly, a thick suspension becoming converted into a jelly. High dilutions of the oleate (e.g. 1 : 10,000 to 20,000 in a broth culture) which are not lethal for pneumococci, have the property of accelerating autolysis of the organisms. This action of sodium oleate is inhibited by the presence of serum in the mixture of soap and pneumococci, but inhibition can be overcome to a considerable extent by the addition of a suitable concentration of boric acid. Soaps of certain other unsaturated fatty acids, e.g. sodium linoleate and linolenate, have an effect similar to that of sodium oleate, but they act both more rapidly and in higher dilution according to the degree of unsaturation of the acid. The inhibitory action of serum on the soaps of the more unsaturated acids could not be overcome, however. A minute concentration of sodium oleate, e.g. 1 : 20,000, when added to a broth culture of virulent pneumococci, so altered the latter after brief contact, e.g. one hour at room temperature, that when the organisms were then separated and washed by centrifuging, they subsequently underwent lysis *in vitro* on the addition of an immune serum (goat v. pneumococcus), and to a less extent under the influence of normal serum. Neither serum had such an effect on untreated organisms. Accordingly, susceptible animals, such as rats, when inoculated intraperitoneally with the ' soaped ' pneumococci to which immune serum had been added subsequently, did not become infected. On the other hand, animals inoculated with pneumococci which had been treated similarly with sodium oleate alone or with sodium oleate followed by normal serum died of pneumococcal infection. The results of Lamar have been confirmed generally, e.g. by Walker (1924), who showed also that pneumococci are highly susceptible to lauric acid, since N/10,240 solution (1 : 50,000) of sodium laurate in water is lethal in

15 minutes. According to Larson and Nelson (1925) virulent cultures of pneumococci are instantly deprived of their pathogenicity by the addition of 0·1 per cent. sodium ricinoleate.

Morgenroth and Levy's (1911) discovery that ethyl-hydrocupreine could cure pneumococcus infection in mice is the first positive evidence of chemotherapeutic action in a progressive general bacterial infection. The use of this substance was suggested by the fact that ethyl-hydrocupreine, subsequently called optoquine (optochin in German), had a therapeutic effect in mice infected with trypanosomes, which like the pneumococcus are dissolved by bile-salts, and also by the reputed beneficial clinical action of quinine in cases of lobar pneumonia. A watery solution of a salt of optoquine base was given at first ; the tolerated dose by subcutaneous injection was 0·5 c.cm. of a 0·75 per cent. solution of the hydrochloride for a 20-gm. mouse, and this could be repeated on 3 or 4 successive days (0·7 c.cm. of the same solution was frequently fatal). Cures were obtained in 50 per cent. of the animals when treatment was begun 5 to 6 hours after intraperitoneal inoculation, which led to death of the untreated controls in 2 to 3 days. The therapeutic effect was found to be greater on employing a solution of the base in oil, prepared by heating, of which the dose employed was 0·4 c.cm. of a 2 per cent. solution, although often less sufficed, e.g. 0·25 c.cm., i.e. half the tolerated dose (Morgenroth and Kaufmann, 1913). Treatment was commenced six hours after intraperitoneal inoculation, when pneumococci were proved to be present in the peripheral blood ; the dose of 0·4 c.cm. was repeated daily for four days. This represents approximately the maximum dosage tolerated, which is, however, higher at warm periods of the year than at the cold seasons (Neufeld and Engwer, 1912 ; Morgenroth, 1912). In these tests the untreated control animals were all dead by the third day. Even when treatment was not begun until 20 hours after inoculation, when organisms were swarming in the blood, a proportion of the animals were cured by three daily injections, each of 0·3 c.cm. of a 2 per cent. solution of the base.

Infections with twelve different strains of pneumococci were found to respond to prophylactic treatment (Gutmann, 1912), and a bile-soluble strain of *Streptococcus mucosus* was also influenced (Levy, 1912). But variations were observed in the susceptibility of different strains and also of the same strain at different times (Morgenroth and Kaufmann, 1913). The therapeutic action of optoquine in pneumococcus infection in mice was confirmed by Boehncke (1913), Moore (1915) and others, while Neufeld and Engwer (1912) by treating guinea-pigs with the drug at the same time as they received an intra-pulmonary inoculation with pneumococci frequently prevented the fatal broncho-pneumonia which results in untreated animals.

It appears that the mode of action of optoquine in the infected animal may be in great part that of an internal antiseptic which acts directly on the micro-organisms, since pneumococci are highly susceptible to

optoquine *in vitro*. But doubtless loss of virulence of the organisms brought about under the influence of optoquine plays an important part in conducing to cure (see Tugendreich and Russo, 1913). Wright and his co-workers (1912) showed that although human serum became lethal to pneumococci after the administration of optoquine (e.g. three hours after 0·5 gm. by mouth for an adult), its opsonic power was not appreciably altered. Similarly Neufeld and Engwer (1912) found that in guinea-pigs the drug caused extracellular destruction of the cocci, but did not lead to phagocytosis. According to Kolmer, Solis-Cohen and Steinfield (1917), however, high dilutions of optoquine and other quinine alkaloids, or the serum of rabbits to which the drugs had been administered, aided the ingestion of pneumococci by rabbit leucocytes *in vitro*.

According to Koch (1920) pneumococci after cultivation on artificial media may lose their sensitiveness to optoquine without losing their virulence. Drug-resistant strains of pneumococci were obtained regularly by a procedure analogous to that which Ehrlich's co-workers had discovered in the case of trypanosomes (Morgenroth and Kaufmann, 1912). Thus the pneumococci were passed through a series of mice which were treated with optoquine in a manner insufficient to produce cure of the infection. Drug-resistance developed rapidly, since after four passages in the course of eight days, a highly resistant strain resulted. The resistant organisms were fully virulent, and the infection which they produced was not influenced by the most energetic treatment with optoquine. It is noteworthy, however, that very vigorous treatment with optoquine in a single passage did not produce drug-resistant organisms. When resistant pneumococci were passed rapidly through a series of animals which received no further treatment, or when they were preserved in the dry state in the spleen and then passed repeatedly through normal mice (e.g. ten times), the drug-resistance persisted for several months at least. Resistance to optoquine was also developed *in vitro* by inoculating fluid medium containing a concentration of the drug which permitted growth, and then, in a series of passages through further cultures, accustoming the organisms to higher and higher concentrations (Tugendreich and Russo, 1913).

The virulence of pneumococci which are being rendered resistant to the drug *in vitro* is liable to diminish (see Jungeblut, 1923). In order to preserve their virulence, the organisms should be passed alternately through untreated mice and through culture medium containing optoquine. In this way, Lewy (1925) raised the resistance to optoquine to 80 or 120 times that of the original strain, and at the same time maintained the organisms highly virulent. The drug resistance thus acquired is manifested *in vivo* as well as *in vitro*. It is specific in the sense that an optoquine-resistant strain does not possess increased resistance to acridine derivatives (Lewy, 1925). The resistance persists for long periods when the strains are cultivated subsequently in media containing no optoquine or when they are passed through untreated animals. On the other hand, when

the organisms are maintained entirely in cultures, treatment with optoquine tends to lead to the development of non-virulent organisms with the characters of non-hæmolytic streptococci. A further hæmolytic modification may, however, be derived from them (Morgenroth, Schnitzer and Berger, 1925). Several procedures may give rise to this series of transformations, but the following is stated to have been highly effective. To 2 c.cm. of a 20-hour serum broth culture were added 2 c.cm. of a mixture of two parts serum broth with one part 20 per cent. watery suspension of a preparation of dried yeast. The whole was incubated for $2\frac{1}{2}$ hours, then one drop was used to inoculate 2 c.cm. of serum broth. After this culture had been incubated for $2\frac{1}{2}$ hours further, its resistance towards optoquine in serum broth was tested *in vitro*. As control a serum broth culture of the same strain of pneumococci was used which had never been subjected to contact with the yeast. Abundant growth of the treated culture occurred in the presence of optoquine 1 : 40,000, whereas a concentration of 1 : 1,280,000 prevented growth of the control, as tested by subculturing after the mixtures had stood for 24 hours at 37° C. The optoquine-resistant cocci were also insoluble in bile ; on intraperitoneal inoculation of these organisms into a mouse, death occurred in 24 hours and a hæmolytic streptococcus was recovered from its body.

According to the views of Morgenroth and his co-workers, treatment with yeast or animal charcoal leaves the organisms with all the usual biological characters of pneumococci, including virulence, but modifies them in such a way that on subsequent treatment with optoquine they tend to give rise to non-hæmolytic, non-virulent streptococci. The latter may then become transformed into hæmolytic virulent streptococci on subcutaneous or intraperitoneal inoculation, or on transference through culture media, or, occasionally, after contact with optoquine *in vitro*. The series of transformations may also occur in the reverse order. The possibility that the cultures may contain latent components or that mixed infections may occur *in vivo* does not appear to be entertained. According to Reimann (1926), however, optoquine changes the usual ' smooth ' pneumococcus into the ' rough ' (R) type, the change being similar to that produced by other agencies. Amzel (1927) found also that after the administration of optoquine to patients with pneumonia, the pneumococci cultivated from the ' pus ' were, at least in part, of the rough type, whereas before treatment they were all smooth.

Instead of the production of resistant strains of pneumococci, the opposite result may follow, namely, the development of strains hypersensitive to the drug. This may be effected by bringing pneumococci in contact *in vitro* or in the body of an infected animal with very minute quantities of optoquine (Schnabel, 1922 ; Schnabel and Kasarnowsky, 1923).

Mice which have been inoculated with pneumococci and treated with optoquine may show later, e.g. after six days or longer, definitely increased resistance to reinfection with the same organisms (Morgenroth and

Levy, 1911); but when in prophylactic experiments cure has been effected after inoculation with minute doses of highly virulent organisms no such immunity develops (Morgenroth and Kaufmann, 1913). The therapeutic action of optoquine and of antipneumococcus serum show marked mutual intensification in guinea-pigs on intrapulmonary inoculation (Neufeld and Engwer, 1912) and in mice (Boehncke, 1913).

In the experiments of Kolmer and Sands (1921) promising results were obtained on treating pleural infections in guinea-pigs and dogs by intrapleural injections of optoquine (1 c.cm. 1 : 500 solution per kgm. of body weight). With optoquine together with Lamar's mixture of sodium oleate and boric acid very good effects were got in guinea-pigs. The animals had been inoculated with 1 c.cm. of a 24-hours broth culture injected into the right pleural sac. A single injection was given into each pleural sac of 0·5 c.cm. of a mixture of equal parts of 1 : 100 optoquine, 1 : 200 sodium oleate and 5 per cent. boric acid up to 24 hours after inoculation, and the animals survived. The untreated control died in about 48 hours.

Stewart (1927) has treated with success pneumococcal meningitis in dogs produced by intracisternal injection of suitable doses of young actively growing cultures of Type I pneumococci. The treatment consisted in lavage, where possible, with saline entering at the cisterna and flowing out at a lumbar puncture, followed by combined intracisternal, intraspinal and double frontal injections of type-specific antipneumococcus serum, with each 15 c.cm. of which 0·75 c.cm. of 1 per cent. optoquine hydrochloride solution was mixed. Sterilization was effected in some animals which were at the initial stage of the infection by two treatments (e.g. 19 and 42 hours respectively after inoculation); in others at a more advanced stage cure resulted after as many as seven treatments, e.g. when treatment was begun 42 hours after inoculation.

In rabbits optoquine appears not to exert either prophylactic or therapeutic action against general pneumococcus infection (Scott, 1914), even when administered continuously by the intravenous method over some hours (Lewis, 1918). This failure is attributed by Scott to the greater activity of rabbit's tissues, especially the liver, as compared with those of mice or guinea-pigs in absorbing or destroying the drug (see also Boecker, 1916). But a local pneumococcal infection of the cornea in rabbits produced by injecting virulent organisms into the substance of the cornea, has been sterilized by applying a watery solution of optoquine either by instillation into the conjunctival sac or by subconjunctival injection (Ginsberg and Kaufmann, 1913). Hence the drug has been successfully employed for the treatment of ulcus serpens in the human subject (Goldschmidt, 1913, 1914).

Sanocrysin, according to Schiemann and Feldt (1926) has little chemotherapeutic effect on pneumococcus infection in mice.

In Lamar's (1911[1&2]) therapeutic experiments rats inoculated intraperitoneally with several lethal doses of pneumococci survived, when,

up to one hour later, they received an intraperitoneal injection of a mixture of sodium oleate, immune serum and boric acid. When the interval between inoculation and treatment was longer the effect of the latter was uncertain. Therapeutic experiments with the soaps of the other unsaturated acids were less successful than those in which oleate was used. In experimental pneumococcus meningitis in monkeys produced by intraspinal inoculation, repeated intraspinal injections of a mixture of sodium oleate, immune serum and boric acid exerted regularly a more powerful action than immune serum alone, and not only prevented the occurrence of infection, but also when administered repeatedly arrested the progress of an actually established infection, and led often to complete recovery, this result being got in some animals even when treatment was begun 18 to 24 hours after inoculation. When the infection is still more or less localized a degree of therapeutic action may be exerted by various other chemical substances (see Browning and Gulbransen, 1919 ; Kolmer and Idzumi, 1920). Boehncke (1913) found that camphor dissolved in oil when injected subcutaneously in mice or rabbits conferred protection against intraperitoneal or intravenous inoculation with pneumococci some hours later.

Reinhardt (1922) investigated the action of various substances in preventing the development of local and general pneumococcus infection in guinea-pigs which had been inoculated by rubbing virulent organisms into wounds in the abdominal skin. The treatment was carried out about half an hour after inoculation, and consisted in washing out the wound with 10 c.cm. or more of a solution of the substance to be tested. The following drugs led to cure in the range of concentrations indicated—trypaflavine (1 : 100 to 1 : 10,000) ; iso-octyl-hydrocupreine bihydrochloride, i.e. vuzine (1 : 300 to 1 : 3,000) ; optoquine (1 : 300 to 1 : 1,000) ; but none of these doses was invariably curative. The general conclusion reached was that of those substances trypaflavine was the most effective and optoquine the least ; phenol (5 per cent.) and tincture of iodine were without any perceptible action, but mercuric chloride (1 : 1,000) prolonged the life of the animals. The controls in which the wounds were washed out with physiological salt solution lived 2 to 7 days. Similar experiments were carried out with mice, which showed that in the latter animals the infection was more difficult to combat, although here also a proportion of cures was obtained.

The Treatment of Pneumococcus Infections in the Human Subject.

This can only be referred to briefly. The powerful direct action of optoquine on pneumococci, as well as the striking therapeutic results obtained with it in infected mice, strongly suggest its use in localized conditions, and, as already stated, pneumococcus ulcer of the cornea has been successfully treated by this drug (see also Bedell, 1920). In lobar pneumonia, optoquine has been fairly widely employed (see the critical review of Laqueur, Grevenstuk, Sluyters and Wolff, 1923 ; also Kolmer,

1926), but agreement as to its value has apparently not been reached. It is obvious that with established pneumonic lesions evidence as to the action of the drug must be obtained from controlled statistical observations concerning the severity of symptoms, date of crisis, degree of bacteriæmia, mortality, &c. Such data on a sufficiently extensive scale, however, are lacking. A toxic effect of optoquine which has been noted in a proportion of cases is disturbance of vision, occasionally amounting to blindness, which may very rarely be persistent. It appears, however, that this complication has occurred mainly after administration of the soluble hydrochloride, and that it may be minimized by avoiding excessive dosage ; thus relatively insoluble forms of the drug should be used, e.g. the base ; no preparation should be allowed to come into contact with free hydrochloric acid in the stomach. Stewart's work on the treatment of pneumococcus meningitis in dogs has shown how difficult such a therapeutic problem may be, although the success obtained affords the indication for a similar procedure in human cases.

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CHAPTER IV. THE GONOCOCCUS.

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With a Section by W. Bulloch, London Hospital.

Micrococcus gonorrhœæ, or as it is commonly termed 'the gonococcus', is the cause in man of gonorrhœa and its complications. The organism is placed by the American Committee in the genus '*Neisseria*' (*Neisseria gonorrheæ*) with the meningococcus, *M. catarrhalis*, and some other organisms.

History.

By W. Bulloch.

Until the middle of the nineteenth century much confusion existed on the nature and varieties of venereal diseases. Syphilis had for long been recognized as a special or specific virulent disease. In May, 1767, John Hunter inoculated himself on the penis with a lancet dipped in matter from a case of gonorrhœa. The symptoms which ensued were undoubtedly those of syphilis, from which he inferred that syphilis and gonorrhœa were manifestations of the same malady. This was not universally accepted, and indeed, as a result of many inoculations in man, was refuted by Ricord (1832). He held that the two diseases were separate, that syphilis was specific, and that gonorrhœa was not; for it appeared possible to contract the disease from discharges other than those in gonorrhœal patients. While, therefore, Ricord did a service in finally differentiating syphilis from gonorrhœa, his views on the latter side retarded progress. As time went on dissentient voices were raised and the general conviction grew that gonorrhœa was also a specific disease. It was in this belief that Donné (1837) and Salisbury (1862), searched for a supposed virus, although it is now known that they were entirely unsuccessful. Working with the new bacterioscopic methods which had been introduced by Weigert and Koch, Albert Neisser (1879) examined the pus from cases of gonorrhœa and found a characteristic micrococcus which he compared to the shape of a roll of bread. It was mostly in pairs or in fours, a fact which Neisser correctly ascribed to its division first in one plane and then in another at right angles to the first. He stained the coccus with methyl violet, and found it in 35 cases of urethral gonorrhœa in the male, in 9 cases of urethritis in the female, in 2 cases of vaginal discharge in raped children, and in 9 cases of ophthalmic blenorrhœa (7 in children and 2 in adults). The coccus was not to be found in cases of soft chancre, hard chancre, buboes, nor in 13 cases of simple fluor vaginalis. From the fact that it

was constantly present in cases of gonorrhœa from different sites and from the fact that no other organisms were found in gonorrhœal discharges, Neisser formed the opinion that the coccus was specific for gonorrhœa. To strengthen this belief, Neisser (1882) attempted to cultivate the coccus on gelatin but was not very successful. He claimed success from at least one culture, but from our knowledge of the gonococcus to-day his conclusion may be doubted. In 1885, E. Bumm, a gynæcologist working in Würzburg, published an exceedingly accurate and indeed classical work on the gonococcus. Every aspect of the organism was most carefully described, and of particular interest and importance were his studies on the histology of the gonococcal process. This he was able to follow in small pieces of conjunctiva removed from 26 cases of ophthalmic blenorrhœa which had lasted from 36 hours to 32 days. The pieces snipped off from the affected conjunctiva were cut in serial sections and stained to show the coccus. Bumm also employed himself in attempting to cultivate the gonococcus outside the body. For this purpose he used a medium consisting of sheep or ox-serum which was stiffened by heat. To increase its nutritive value he finally added some human blood-serum obtained by expression of the placenta, and the human serum having been placed on the surface of the stiffened animal serum, the whole was subjected to a further heating to set the human serum. On this medium he obtained some undoubted growths of the coccus of Neisser and he accurately described the growths as they appeared to the eye and to the microscope.

From a pure culture raised from a case of ophthalmic gonorrhœa he inoculated a small quantity into the urethra of a healthy woman previously proved to be free from any suspicion of the disease. The result was the production of a typical and rather severe gonorrhœa, which exhibited the gonococcus in the secretion. Bumm thus proved for the first time the specific pathogenic properties of Neisser's coccus. Of subsequent workers special praise must be given to Ernst Wertheim (1891[1]), a gynæcologist working at the time in Prague. He invented a simplified method of obtaining pure cultures of gonococcus on plates and he it was more than anyone else who demonstrated the dominant part played by it in inflammatory diseases of the female genital organs. Like Bumm, he collected human blood-serum from the expressed placenta, but instead of heating it so as to form a solid blood-serum he inoculated it with dilutions of gonorrhœal pus and added it to a nutrient peptone agar which was kept at 40° C. in readiness. This serum agar medium was rapidly poured into plates and was found to give more luxuriant growths of the organism than had been obtained by Bumm. In a second and much larger paper on the ascending gonorrhœa in the female, Wertheim (1891[2]) gave a very complete account of his technique and its importance in the diagnosis of gonorrhœa. He also, by means of inoculation of pure cultures in the urethra, produced typical clinical gonorrhœa in five persons, and he demonstrated the presence of the coccus in salpingitis, oophoritis, perimetritis and parametritis. Our knowledge of gonococcus was also greatly

increased by the researches of Finger, Ghon and Schlagenhaufer (1894). Among other things they showed that persons who had had gonorrhœa were capable of being reinfected by pure cultures of gonococci from different sources, and for the purposes of an anatomical study of the histogenesis of the blenorrhagic process they inoculated eight persons suffering or moribund from pneumonia, consumption or other diseases. Three of these cases showed typical gonorrhœa, the negative results being attributed to the existence of high fever at the time inoculations were made. W. B.

Relation of the Gonococcus to other Micro-organisms.

The outstanding features of the gonococcus are its strictly limited parasitism, its very definite nutritional requirements in culture, and its susceptibility to external influences. It may, therefore, be regarded as a 'delicate' micro-organism in that it is difficult to cultivate, but it is none the less tenacious of life in its natural habitat. It resembles the meningococcus to some extent in these qualities, and, indeed, there is a close similarity between the gonococcus and the meningococcus, in form, in staining properties, and in cultural requirements, while both tend to undergo autolysis with considerable rapidity. Moreover, it is not always easy to obtain clear-cut serological differentiation between these two micro-organisms, but the most striking similarity between them is that both are strict parasites, are parasites of but one animal species—man, and both tend to invade special areas of the body. The meningococcus in the majority of cases is found only in the nasopharynx and meninges, producing the clinically defined disease on invasion of the latter, while the gonococcus in the majority of instances invades only the genito-urinary or conjunctival mucosa; although both do give rise to general infections with involvement of various organs, such generalized infection is not the outstanding feature of their disease-producing activities. It must be noted, however, that the meningococcus may occasionally be found giving rise to infection of the genitals and the gonococcus may give rise to a meningitis. Thus, according to Reuter (1905) and Schottmüller (1905), metastatic meningococcal infection of the genitals is not an unknown complication of cerebrospinal fever, while occasional cases of gonococcal meningitis have been observed. Several cases of presumed gonococcal meningitis have been described, but in many of these the investigation of the micro-organism was not sufficiently complete to permit it to be classed unequivocally as the gonococcus. Smith (1922) has, however, given a full account of a case of meningitis in which the gonococcus was certainly the causal organism.

The similarity of the gonococcus to the meningococcus even in respect of serological relationship is striking, and attention has been called to it by numerous authors. The following examples may be cited to show this similarity between the two micro-organisms at present under consideration. Bruckner and Cristéanu (1906) in several publications called attention

to this similarity both in respect of agglutination and precipitation reactions. Martha Wollstein (1907) amplified these observations of Bruckner and Cristéanu by making a similar comparison between meningococci and cultures of Gram-negative diplococci isolated from cases of vaginitis in children, and reached the same conclusion. There are, however, two points in this communication by Wollstein that are worthy of note : in the first place the carbohydrate fermentation reactions of her cultures were not those typical of the gonococcus, there being some acid-production in presence of maltose, and secondly, the titre of the sera which were used in the tests was somewhat low.

Nicolle, Jouan and Debains (1919) observed after treatment of suspensions of both micro-organisms with acid and subsequent neutralization with sodium hydrate—a modification of a technique described by Porges (1905) and used by him in the investigation of the agglutinability of bacteria—a marked serological group relationship between the gonococcus and the parameningococcus B of Dopter, which is said to differ serologically from the four types of meningococci described by Gordon (1916).

Gordon (1921) in a personal communication informed the writer that from the standpoint of agglutination his Type IV meningococcus exhibited a particularly close relationship to the gonococcus ; while Tulloch (1922) showed a similar relationship between the gonococcus and a Type I (Gordon) strain of meningococcus.

There is, therefore, a fairly close serological relationship, at least so far as *in vitro* tests are concerned, between gonococci and meningococci, but it seems probable that the group relationship which appears to exist between them depends upon peculiarities of the strains examined and bears but little relationship to the specific type characters of the two micro-organisms. The obvious explanation of the discrepancies in the results quoted from these various authors is, then, that group relationship between meningococci and gonococci is a factor peculiar to each strain, and not of any serological ' Type ' of either.

On the other hand, Vannod (1906) and Torrey (1907) find that there is, notwithstanding this similarity, a marked difference in the degree to which these micro-organisms are agglutinated by their respective sera, and that group reactions are especially common when the sera employed in the tests are of low titre.

On applying the absorption of agglutinins test it can be readily shown that there is but little difficulty in differentiating between strains of the gonococcus and strains of the meningococcus, although such differentiation is rendered laborious owing to the multiplicity of the serological types of the two micro-organisms under consideration. Similar findings are obtained when the complement-fixation reaction is employed for the differentiation of the gonococcus from the meningococcus.

On animal inoculation, too, the lesions produced by these two micro-organisms are comparable. Neither of these cocci grow when inoculated

into experimental animals, and the inflammatory disturbance resulting from their inoculation is apparently due not to invasion, but to the liberation of toxic products from the broken-down organisms of the inoculum.

The relationship of the gonococcus to other Gram-negative diplococci is more remote. These do not present such difficulty of cultivation nor demand such epicurean nutritional conditions as are required by the gonococcus so strikingly or by the meningococcus less markedly. Some of these other Gram-negative cocci are, however, of considerable practical importance, as their presence in the secretions of the genital organs may lead to some difficulty in diagnosis. This will be discussed later in dealing with the laboratory diagnosis of gonorrhœa by microscopical methods.

Morphology.

Features of typical gonococci. The gonococcus is seen in its most typical and apparently most stable form in acute exudates such as the urethral pus of (acute) gonorrhœa. In such material it seldom exhibits the great variation of shape, size or staining properties which it shows in smear preparations from cultures.

The finer points in the morphology of the micro-organism are best demonstrated in thin films of pus rapidly dried and then fixed by heat, the preparation being stained thereafter by a simple stain such as carbol thionin blue or aqueous solutions of methylene blue. Very pleasing preparations showing a minimum of distortion are obtained with Pappenheim's stain (vide chapter on staining technique, Vol. IX). Fixation by heat is advised, as fixation by alcohol is liable to cause some degree of shrinkage, so that the cocci then appear unduly small. A simple stain is preferable to special methods, such as that of Gram, when the finer points in the morphology of the micro-organism are to be demonstrated. It must, however, be emphasized that Gram's method is of paramount importance in diagnosis.

The micro-organism is a kidney-shaped diplococcus with the hila of kidneys apposed, and roughly one-fifth of the total area covered by a pair of cocci consists of the space between its two constituent units. The average size of the organism is 1μ long by $0{\cdot}6\mu$ broad. These detailed features of the most typical form of the gonococcus are important, especially in the examination of smears of conjunctival pus.

Staining properties. The gonococcus is definitely Gram-negative and in both smears of exudates and preparations made from cultures, when stained by any of the recognized modifications of Gram's method, it loses the Gram stain and takes up the counterstain. Gram-positive cocci, when phagocytosed, may lose their Gram-positive character, but, with care, little difficulty should arise from this, as usually such cocci within the phagocytes are surrounded by a vacuole and do not have the characteristic 'two-kidney' appearance of the gonococcus. Sometimes, too, organisms may be so situated in an exudate that penetration of the reagents used in

the method is difficult, and in such circumstances the gonococcus may retain the Gram stain to an unexpected degree, thus appearing to be Gram-positive.

Distribution in morbid exudates. In the early acute case of gonococcal infection the micro-organisms are, for the most part, situated outside the pus cells of the exudate and lie in clumps of varying size either free, or applied to epithelial cells. Later, in the average case, when the discharge has become more definitely purulent, the cocci are found apparently within the pus cells, and this intracellular disposition of the micro-organisms is an outstanding feature of gonococcal pus in the later phases of acute gonorrhœa. It is especially noticeable that not all the pus cells are equally active as phagocytes, and it is usual to find that a few cells per microscope field contain very large numbers of the cocci, while the majority contain none. In the later chronic stages of gonorrhœal infection of the genito-urinary tract the distribution of the organism in the exudate is irregular and the characteristic arrangement which is so marked a feature of the acute phases of the infection is lost.

Morphology in culture. The form of the gonococcus in smears made from cultures is very variable, as the characters of the cocci are much modified by the nature of the culture medium and by the time and temperature of incubation. The organism is seen in its most typical form when cultivated for about 6 hours at between 36 and 37° C. on a suitable medium. When thus examined its morphology is indistinguishable from that seen in morbid exudates, but in older cultures—even in 24 hours at 37° C.—if the growth has been luxuriant one finds a mixture of degenerated cocci and normal cocci. The reason for this appears to be that in certain cultural conditions the gonococcus rapidly undergoes autolysis, and if a single colony is examined the centre of the colony may be found to consist of degenerate forms, while the spreading margin consists of kidney-shaped normal diplococci. Autolysis can proceed to such a degree that the organisms are almost entirely disintegrated, the preparation having the appearance of mucus and differing therefrom only in that it is somewhat granular. Amongst this detritus of degenerated cocci are seen numbers of ' giant ' forms, which may be several times the size of the ' normal ' coccus. They stain feebly, and often each unit of the ' giant ' diplococcus contains a deeper staining granule. The giant cocci and also others of normal size staining feebly or possessing granules may be regarded as involution forms. It must be emphasized that the appearance of these degenerated forms in the culture depends upon the nutritional qualities of the medium on which the culture is made, the time and temperature of incubation, and the strain which is cultivated. All strains do not undergo autolysis with equal rapidity when cultivated under identical conditions. According to Jenkins (1921) if the cultures are incubated at 35° C. the process is much delayed.

Special morphological features. Apart from the characters already dealt with the gonococcus exhibits no special features. It is non-motile

and is certainly devoid of spores and flagella. The question whether it has or has not a capsule is of minor importance, but not infrequently there appears to be a clear zone around the cocci, and by some this has been interpreted as evidence of a capsule. Thus, according to Israeli (1921) this capsule can be demonstrated by the use of Hiss's method slightly modified. Preparations are fixed by heat, are stained with boiling 5 per cent. aqueous solution of fuchsine, washed with 20 per cent. solution of copper sulphate, and dried with filter paper.

Cultivation.

It appears advisable to deal with this matter from a purely practical view-point, and the special nutritive requirements of the organism are here discussed so that on consulting the technical section of this work the reader may appreciate the special precautions to be taken in preparing culture medium for growing the gonococcus.

Like all micro-organisms the gonococcus demands certain conditions of temperature, moisture and oxygenation, and also certain nutrient qualities and a fairly restricted range of reaction of the culture medium if satisfactory growths are to be obtained. Indeed, it is somewhat exacting in respect of all these requirements, and even when the most satisfactory culture media are employed one meets with occasional strains which, in primary culture at least, are difficult to cultivate.

Most of the media described in the earlier literature were compounded empirically, and it has only been recently that the various factors which make for stimulation or inhibition of growth have received adequate attention from investigators.

Temperature of Incubation.

For satisfactory growth the gonococcus demands a temperature approximating that of normal mammals and, although in subcultures some strains may grow more or less satisfactorily at temperatures from 26 to 39° C., primary growths are obtained only over a much more restricted range. According to Jenkins (1921) the optimum temperature is somewhat below 37·5° C., the most luxuriant growths being obtained at 35 to 36° C. Not only is growth more rapid and luxuriant at 35 to 36° C. than at 37·5° C., but cultures incubated at the lower temperature do not undergo autolysis to the same extent as when incubated at the higher temperature. This observation of Jenkins, which has been verified by the writer, is of considerable importance ; for not only does incubation at the lower temperature assist in the isolation of a micro-organism which is difficult to deal with under the best of circumstances, but it also leads to the development of a type of colony which I believe to be more natural than that usually described. The edge of the colony is more regular and exhibits crenations to a less degree than the colonies usually described which are regarded as so characteristic of the gonococcus.

Moisture.

The gonococcus is remarkably susceptible to variation in the moisture of the surface upon which it grows and the atmosphere in which it is incubated. It may be definitely stated that attempts to cultivate it on solid medium which is devoid of condensation water will fail, no matter how satisfactory that medium may otherwise be. Condensation water should, therefore, be present in sloped agar tubes and in the lids of Petri dishes of medium which is to be used for the cultivation of the gonococcus, and failure to observe this simple precaution increases to an unexpected extent the difficulties which are encountered in cultivating the micro-organism. In order to maintain a moist condition while incubation is proceeding the writer employs a glass jar of a size suitable to hold tubes and plates. The jar is covered with a plate luted on with resin ointment, and in the bottom is placed a pad of glass wool wet with a solution of brilliant green. The same apparatus is used for plates, these being stacked upon one another, the lowest plate being supported on a tripod of glass rod to keep it clear from the pad of glass wool. This apparatus is always kept in the incubator so that it is already warm when inoculated tubes are put into it. A similar procedure is advised by Cook and Stafford (1921).

It is also wise to arrange that the medium itself be sufficiently soft, for a hard-surfaced agar is less satisfactory than one whose surface is soft. It is for this reason that cultures on semi-solid agar as advised by Torrey and Buckell (1922), are often successful when cultures on medium otherwise the same, but containing a higher percentage of agar, fail. A useful method of preparing agar having a surface from which colonies can be picked off or cultures washed off to prepare a vaccine, and yet soft enough to ensure that growth will not be unduly inhibited, is described by Jenkins (1921).

Reaction of Medium.

The gonococcus demands for satisfactory growth that the hydrogen-ion concentration of the medium upon which it is grown should be within a certain range. At one time it was thought that this range was narrow, and much work has been done to determine the optimum hydrogen-ion concentration. Opinions have differed greatly on this matter, and it appears that *provided the medium is otherwise suitable the range of reaction over which fairly satisfactory growths can be obtained is considerable.*

According to Cole and Lloyd (1916) the mean optimum reaction is pH 7·6 with a possible range, in certain media, between pH 6·5 and 9·1, but with less suitable media the range was restricted to between pH 7·5 and 7·8. A range of between pH 7·4 and 7·6 was arrived at by Erickson and Albert (1922). Torrey and Buckell (1922), using a semi-solid agar, give the range as pH 5·8 to 8·2 with an optimum zone for growth between pH 6·4 and 7·7, and an optimum for viability at about pH 6·3. It must be understood that some variation of optimum reaction may exist, and if the medium is, from the nutritive point of view, less

satisfactory, the range becomes restricted. It is thus difficult to define an optimum reaction for all circumstances, but it may be considered that between pH 7·3 and 7·6 will satisfy the majority of strains.

Source of Nitrogen in Culture Medium.

Neither pure proteins (Bainbridge, 1911) nor even peptones (Sperry and Rettger, 1915 ; Rettger, Berman and Sturges, 1916) seem to be directly available for bacterial metabolism, and this suggested to Cole and Lloyd (1916) that free amino-acids are probably important constituents of all culture media, and are of special significance in the growth of micro-organisms whose cultivation presents difficulty. The introduction of the ' trypamine ' media of these authors represents a solid advance in the investigation of the cultural requirements of the gonococcus.

Further investigation, however, by McLeod and Wyon (1921) showed that in certain concentrations some amino-acids may inhibit the growth of pneumococci, streptococci, and meningococci, and Torrey and Buckell (1922) found the same to hold good for the gonococcus, and in place of adding an amino-acid to their culture media in the form of trypamine they employed ' Difco ' peptone in concentration of 1·5 per cent. The growth-stimulating and growth-inhibiting qualities of amino-acids and also of certain commercial peptones, however, vary, and McLeod, Wheatley and Phelon (1927) showed not only that certain amino-acids favour, while others inhibit, the growth of the gonococcus, but also that some exert a favouring action in low concentrations and an inhibitory action in higher concentrations. They, therefore, group the amino-acids investigated into three categories :

Group I in high concentrations produce constant inhibiting effects, but in low concentrations may favour growth of certain strains of gonococci, especially when the medium contains a considerable quantity of blood (glycine, tryptophane, phenyl-alanine, cystine, asparagine).

Group II neither exhibit constant inhibitory effects nor stimulate growth (histidine, arginine, lysine, and glutamic acid).

Group III may inhibit growth in high concentration, but favour it in low concentration even in the presence of a *small* quantity of blood. When in high concentration these favour the growth if 0·5 per cent. or more blood is present in the media (alanine d., aspartic acid and taurine).

These investigations of McLeod and his associates afford the most satisfactory explanation of the difficulties encountered in preparing culture media for the growth of the micro-organism under consideration.

Oxygen Requirements.

The gonococcus is an aerobic micro-organism ; on inoculating by stab tubes of semi-solid agar, growth takes place best upon the surface and only relatively slight development occurs in the track of the needle.

Nevertheless, several authors regard the micro-organism as being relatively anaerobic in that they find that better growths are obtained under conditions of reduced oxygen tension. Thus Ruediger (1919), Swartz (1920), Wherry and Oliver (1916), Herrold (1921), and Hermanies (1921) have all employed one method or another of reducing the oxygen tension, while the cultures were being incubated ; while Chapin (1918) achieved the same end by exposure to carbon dioxide. The writer essayed several of these methods, but, in common with Torry and Buckell, Cook and Stafford, and Erickson and Albert, failed to obtain growths more easily, or of greater luxuriance, at reduced than at normal oxygen tension. It is, as Torrey points out, worthy of note that all the methods designed for reducing oxygen tension when cultivating the gonococcus also ensure the retention of moisture in the culture tubes.

Growth-stimulating or ' Accessory Factors ' in the Medium.

The gonococcus, like the hæmoglobinophilic bacilli and some other microbes, demands the presence of certain nutritive factors whose precise characteristics are difficult to define. Cole and Lloyd (1916) refer to these factors as ' growth hormones '. They consider two of these to be important for the growth of the gonococcus : viz. ' A ' present in red cells, readily absorbed, relatively thermo-labile and of special significance in determining the initial phases of growth, and ' N ' present in tissues, relatively stable, not readily absorbed, and useful for producing luxuriant subcultures.

According to Jenkins (1924) the accessory factors present in *serum* are also two in number, and of these one, which is destroyed by exposure to temperatures in excess of 65° C., appears to be necessary for primary growths ; while the other, which is more heat resistant, is necessary for the propagation of the coccus even in the case of old stock strains which have been in artificial culture for some time. Jenkins concludes that the first is present in blood but not in tissue, while the second is present both in blood and tissue.

The nature of the growth-stimulating accessory factors required for the cultivation of the gonococcus has been regarded from another viewpoint by McLeod, Wheatley and Phelon (1927). According to these investigators whole blood is more adequate than serum or washed red cells, and they state categorically that blood heated to 55° C. is superior to unheated blood. It is worthy of note that Martin (1911) probably appreciated this fact, for, in describing the use of the culture medium which he elaborated he specifically states : ' On the surface of each sloped tube three or four drops of sterile heated (57° C. for 1½ hours) human serum are run '. In common with other observers, McLeod and his associates find that heating of the blood to 100° C. causes marked deterioration to the accessory factors.

The most important information of a practical nature obtained by McLeod and his associates is that the addition of blood, and preferably of blood heated to 55° C., eliminates to a considerable extent the

growth-inhibiting properties of certain amino-acids. As these amino-acids may be present in commercial peptones the need for the addition of blood to any medium designed for the primary culture of the gonococcus is self-evident.

Effect of Electrolytes, Sugars, Glycerin and Certain Other Substances.

Electrolytes. Optimum results are obtained when the salts present in the medium are the same as, and approximately correspond in quantity to, those which can be extracted from mammalian tissue. Thus the addition of the salts of Ringer's or Tyrode's solution to blood peptone medium gives better growths of the micro-organism than when the same medium is devoid of these salts. In this connection it is to be noted that, as shown by Martin (1911) and emphasized by Thomson (1918), the addition of 1 per cent. of disodium hydrogen phosphate to media is advantageous, apart from any special action which it may exert in aiding the growth of the gonococcus ; for it is a buffer salt and so its presence simplifies the process of neutralization by stabilizing the reaction.

Glucose. Opinions differ as to the effect of glucose upon cultures of the gonococcus. According to Cole and Lloyd, the addition of this sugar to the extent of 1 per cent. in their media does not favourably influence growth and does tend to lessen the viability of the cultures. On the other hand, Thomson (1918 and 1923) is of opinion that the addition of 2 to 3 per cent. of glucose to his medium results in a more luxuriant growth than when this sugar be omitted.

Glycerin. Glycerin 2 to 3 per cent. is contained in Von Wassermann's (1897) pig serum neutrose agar, and several later authors have described media containing this substance, but opinions as to its value for enhancing the growth of the gonococcus are divided. There does not seem to be any definite evidence that it is itself of value as a nutrient or as a growth stimulant, but it may in certain circumstances modify the physical qualities of the agar jelly and thereby improve its cultural qualities.

Egg, milk, urine and urea. Besredka and Jupille (1913) describe a simple fluid egg medium and there is no doubt that it is fairly satisfactory. Its disadvantage is that it is not clear. The nutrient value of an agar is very greatly enhanced when it is cleared with *whole egg*, one egg being used for each 500 c.cm. of medium. The same is true of the addition of milk whey, which, indeed, is the main constituent of the medium employed by Weil and Noiré (1913).

The addition of urea and urine has been suggested by several authors, but there is no evidence that the presence of these substances in a medium exerts any beneficial influence.

Taurine (*amino-ethyl-sulphonic acid*). According to Gordon (1926) the addition of taurine to culture media from 0·1 per cent. to 0·4 per cent. leads to an increase both in the size and numbers of colonies, although, as noted by Koser and Rettger (1919) taurine is inadequate as the source of amino-nitrogen in culture media. It is, therefore, a little difficult to

understand how taurine acts, but Gordon suggests that its growth-stimulating activity is related to its power of reducing the surface tension of media.

Nucleic acid of animal origin, protamines and histones. According to Lambkin, Dimond and Robertson (1927) the addition of animal nucleic acid, protamines and histones to culture media results in a considerable increase in the nucleo-protein content of gonococci cultivated upon them. It is stated that this is accompanied by morphological changes in the coccus, notably the development of metachromatic granules.

Preparation of Culture Media for Primary Culture.

The technique for the preparation of media suitable for growing the gonococcus is dealt with in the technical section of this work (Vol. IX) and the reader is referred thereto for the necessary information.

The fact that a large number of authors have described media for this purpose which are satisfactory to themselves would suggest that the difficulties in growing the gonococcus are considerable but capable of being overcome by many apparently unrelated manœuvres. Whereas some authors pin their faith to media of great inconvenience and complexity, others are able to obtain copious growths on a plain serum agar such as was originally used by Wertheim.

Cultural Characters.

In primary culture. When the medium is suitable and the inoculum contains a moderate number of gonococci, growth develops as discrete colonies which within 24 hours may occasionally attain a diameter of 2 mm. The outline of the colony varies somewhat. It may be almost perfectly circular, but more frequently there are indentations giving a slightly scalloped margin, often with fine radial striations. The surface is moist, the colour grey, and the colony is elevated above the surface of the medium. The appearance is often very like that of a colony of the meningococcus and might well be described as a plano-convex lens, but more often the surface is less regular and may give the impression of having a central umbo and a slightly rolled overspreading margin. The most variable feature of the colony is its degree of transparency, the growths on the whole being more transparent when the medium is on the alkaline side and less transparent on the acid side of pH 7·6. If a single isolated colony is so situated that its growth is not interfered with by contiguous colonies, it may, on incubation for several days, attain a diameter of 15 mm. or more, and such colonies exhibit the features already described, usually, however, with considerable modification and exaggeration. In these large colonies radial markings are conspicuous and concentric markings are also seen, while the scalloping of the margin is an outstanding feature of the growth. There also develop in such old colonies peculiar opaque patches—areas of super-growth. Blair Martin

(1911) gave a very full description of them, and attention has also been called to them by other authors, notably by Hermanies (1921). Microscopical examination of these giant colonies is interesting, for preparations made from the central portion of the growth show only autolysed cocci whose outline has disappeared, while similar preparations made from the periphery usually show a majority of typical diplococci, staining well and having clearly defined outlines.

If the inoculum contain very large numbers of viable cocci the growth may be confluent, but in other respects exhibits the characters of the individual colonies.

The consistency of the growth is peculiar, having a mucoid quality which is readily appreciated when a colony is picked off the surface of the medium.

In secondary cultures. The secondary cultures of the gonococcus resemble the primary growths, but, on the whole, tend to develop more rapidly, so that if a rapidly growing culture is inoculated on to fresh medium which has been warmed to blood heat, a good growth is often obtained after about 6 hours incubation at 36° C. If a discrete colony in secondary culture is incubated over a prolonged period it exhibits radial markings and the papillation seen in old cultures in primary growth. Atkin (1925) suggests that there is a relationship between papillation of such colonies and their serological reactions.

Cultural Reactions.

Carbohydrate fermentation. The consistency of its carbohydrate reactions constitutes an outstanding feature of the gonococcus, and these are, therefore, of considerable diagnostic significance.

In conducting fermentation tests it is advisable to employ a solid rather than a fluid medium, for this permits of easy demonstration that growth is taking place, while contaminations, which are liable to occur in all media enriched with serum, are readily observed. Any of the media described, to which have been added 1 part in 10 of a 10 per cent. solution of the carbohydrate to be tested along with a suitable indicator can be used. A very suitable indicator is brom cresol purple. The writer always employs semi-solid agar, the requisite quantity of carbohydrate and indicator being added when required and the mixture sterilized at 100° C. for 15 minutes.

The carbohydrates of special value for diagnostic purposes are glucose and maltose, acid being produced in media containing the former, but not in media containing the latter; indeed, on prolonged culture—3 to 4 days at 37° C.—in maltose medium there appears to be a slight intensification of the alkalinity of the medium. Acid is also produced by the gonococcus when suitably cultivated in presence of galactose, but in presence of any carbohydrate the amount of acid produced is slight, and control tubes of the uninoculated medium used for the tests must be incubated along with the inoculated tubes for comparison.

Gelatin cultures. The gonococcus does not reproduce at the temperature of the gelatin incubator, but quite satisfactory growths can be obtained at 37° C. in gelatin media of the same nature as the agar media already described. By planting the tubes in cold water after incubation at 37° C. the gelatin-liquefying properties of the micro-organism can be studied : the gonococcus does not liquefy gelatin. In this connection it is worthy of note that Turro (1894) claimed to have cultivated the organism in acid gelatin, but this observation has not been verified.

Broth cultures. The gonococcus will grow in broth enriched with serum or blood, but the cultures are seldom luxuriant, and growths in fluid media are not in any way characteristic.

Cultures for special purposes. According to Lambkin, Dimond and Robertson (1927) the gonococcus, when cultivated in media rich in animal nucleo-protein, develops ' polar-bodies ' which appear to be metachromatic granules attached to the cocci, for even washing a heat-fixed film with water is said to cause their removal. Cultures containing these so-called metachromatic granules are said to be of special value in the preparation of vaccines for therapeutic purposes.

The addition of crystal violet to culture media in order to restrain the growth of Gram-positive micro-organisms. In cases where secondary infection with Gram-positive micro-organisms, notably *Staphylococcus albus* and diphtheroid bacilli, has occurred, a condition liable to be encountered in the acute exacerbations of chronic gonorrhœa, the isolation of gonococci is rendered difficult. If the medium is not sufficiently moist, the gonococci do not readily grow, and if it is sufficiently moist the colonies of other micro-organisms are liable to spread. In such circumstances some assistance is derived from the addition of crystal violet to the medium. The dye is added to agar in the form of a sterile solution at the same time as enriching fluid—blood or serum—and it is well to employ a series of dilutions corresponding to ·0001, ·00005, ·000025, ·00001 gm. per 10 c.cm. of media. The range of dilutions is necessitated by the fact that strains of gonococci vary considerably in their resistance to the dye. It is only occasionally that the technique suggested proves of practical value.

Autolysis of the Gonococcus.

The gonococcus is very liable to undergo autolysis, but the mechanism of this degeneration is, so far, ill understood. The process may be very rapid, and even in 24-hour cultures at 37° C. on certain media, the majority of the cocci already show signs of the change, as evidenced by failure to stain, variation in size, and irregularity of outline. According to Wollstein (1907) who repeated with the gonococcus the experiments of Flexner (1907) on the meningococcus, the process is dependent upon an autolytic enzyme which is destroyed by exposure to a temperature of 65° C. for 30 minutes. The influence of the electrolytes in which suspensions of the micro-organism are, and the reaction of the media,

play important parts in this process, which proceeds more rapidly at 37° C. than at lower temperatures. Wollstein (1907) states that when solutions of sodium chloride were used more gonococci remained viable at 7° C. than at 37° C. when the suspensions were strong, and fewer in the case of weaker suspensions. In Ringer's fluid, on the other hand, the survival of the concentrated suspensions was more marked at 37° C., and of the weaker suspensions at 7° C. The interpretation of these observations presents difficulty, for, in the experiments quoted, two separate factors have to be considered, viz.: viability and autolysis, the one of which is not necessarily an index of the other. The viability is partly conditioned by the antagonistic action of the calcium ion to the sodium ion, the latter of which is toxic for micro-organisms, as was shown in the case of the meningococcus by Shearer (1919), so that Wollstein's microscopical examinations give possibly a truer index of the influence of salts upon autolysis than do her cultural investigations. Briefly, her findings were that suspensions kept in the ice-chest showed much less disintegration than those kept at 37° C. There was less disintegration in weak than in strong suspensions and less in suspensions made in Ringer's solution than in those made in saline.

There appears to be no doubt that the process, whatever its nature, occurs with greater rapidity in alkaline than in neutral fluids, while it is definitely inhibited by acid. Thus, according to Thomson (1923), suspensions made in 0·6 per cent. solution of acid sodium phosphate do not undergo autolysis.

According to Warden (1915), chemical analysis of the micro-organism shows it to contain a considerable quantity of fatty acid, there being 0·27 gm. of fatty acid by weight in 0·5 gm. of air-dried gonococci, and he regards autolysis of the micro-organism as being mainly a process of hydrolysis. Thomson also inclines to the view that the process is hydrolytic, but lays stress upon the part played by hydroxyl ions in inducing the change.

The evidence goes to show that true autolysis of the gonococcus is caused by an intracellular enzyme which is most active over a range of hydroxyl concentrations on the alkaline side of absolute neutrality, but owing to the gonococcus being readily dissolved by alkali alone, it may be difficult to determine where the true autolytic process ends and chemical disintegration by alkali begins.

Serological Reactions.

As with other micro-organisms, serological investigation of the gonococcus is carried out with a view, on the one hand, to differentiate it from all other micro-organisms, and, on the other hand, to differentiate strains of gonococci among themselves. The types of serological investigation which have been mainly used for this purpose are agglutination and complement fixation, and as agglutination is

on the whole the more satisfactory of these two reactions in that it is more easily interpreted, it will be dealt with first.

Preparation of Suspensions of Gonococcus for Agglutination Tests and for Immunization of Rabbits.

Cultures destined for agglutination tests are, after isolation, subcultured on a suitable medium, and after 24 hours at 37° C. the growth is scraped off with an iron loop and transferred to a glass mortar, triturated, suspended in saline and heated in a water-bath to 60° C. for 30 minutes. Carbolic acid, 5 per cent. solution, is then added in sufficient quantity to make the final concentration of that reagent 0·5 per cent., and the suspensions are finally standardized to contain 8,000 million cocci per c.cm. For agglutinations these standard preparations are used diluted with an equal bulk of 0·5 per cent. carbolic saline, and for absorption tests 2 c.cm. of the 8,000 million suspension are used for absorbing a definite number of ' units ' of the agglutinating serum.

Primary cultures can be used for agglutination and absorption of agglutinins tests, but on the whole these do not react to such high titre as do suspensions prepared from subcultures. Cultures destined for immunizing rabbits are prepared in the same way, but the strains used should be subcultured for two or three generations in rabbit plasma (if rabbits are to be inoculated) before being cultured in bulk, on the same medium, for preparing the actual suspensions used for inoculation. In immunizing the rabbits a suspension of 16,000 million cocci per c.cm. is used and inoculation is made by the intravenous route in doses of 0·25 c.cm., 0·5 c.cm., 0·75 c.cm. and 1 c.cm., an interval of 2 days being allowed to elapse between each inoculation. The serum is sampled on the fifth day after the last injection and if its titre is 1/1,600 or more, the animal may be bled out under ether anæsthesia.

In view of the statements made by Hermanies and by Gordon (personal communication) that the gonococcus not infrequently agglutinates in presence of normal rabbit serum, the rabbits which it is proposed to use for the preparation of agglutinating serum should be tested out in dilution of 1/10 to 1/40 before the process of immunization is commenced. It has been the personal experience of the writer that young recently isolated cultures of the gonococcus do not agglutinate in presence of normal rabbit serum.

Specificity of Agglutination of the Gonococcus.

According to Bruckner and Cristéanu, anti-gonococcus (horse) serum does not serve to distinguish between gonococci and meningococci. A similar observation was made and personally communicated to the writer by Gordon (1921), who found that there was a special tendency for Type IV (Gordon) meningococci to agglutinate in presence of anti-gonococcus (rabbit) serum. Warren (1921) made a similar observation in the case of Type II (Gordon) meningococci, while the writer (1922) noted a similar

relationship in the case of Type I (Gordon) meningococcus, rabbit serum being used by both these authors. Nicolle, Jouan and Debains (1919), using horse serum, found that the most marked group relationship between the gonococcus and the meningococcus was exhibited, after treatment of the suspensions with acid, by the parameningococcus B of Dopter, which is said to differ serologically from the type meningococci described by Gordon.

The conclusions to be drawn from these observations are, in the first place, that agglutination alone does not serve adequately to differentiate between all gonococci and all meningococci; and secondly, that the obvious explanation of the discrepancies in the findings is that the group relationship between gonococci and meningococci is a factor peculiar to each strain, and is not a factor of any serological ' type ' of either. It is, however, easy to exaggerate the difficulty introduced by the group relationship of gonococci with meningococci, and, in common with Vannod (1907), Torrey (1907), and Elser and Huntoon (1909), the writer is of opinion that with properly controlled tests and using active antiserum obtained from the rabbit, most gonococci can be differentiated from meningococci by direct agglutination. Although agglutination may fail to differentiate between the two micro-organisms under consideration, the absorption of agglutinins test is reliable for this purpose.

Agglutination and Absorption of Agglutinins as Means of Classifying Gonococci.

Recent work. A review even of recent work dealing with the serological classification of gonococci indicates that considerable diversity of opinion exists concerning the relationship which various strains of the gonococcus bear to one another, and the relative frequency with which any given serological type of that organism is associated with gonorrhœa has so far received but little attention in this country, although Hermanies (1921) and Torrey and Buckell (1922) have made an extensive examination of this kind in America.

Earlier researches by Torrey (1907–8) and by Teague and Torrey (1907), suggested that the gonococcus comprised a number of micro-organisms differing *inter se* in their antigenic properties in respect of agglutinating, complement fixing and bactericidal antibodies. In the series examined by Torrey such diversity was noted that it appeared improbable that subgroups of the gonococcus could be clearly defined or the whole group identified by serological methods, but Torrey's series consisted only of a small number of strains and no attempt was made to determine how frequently any of the types was responsible for the causation of disease. Likewise, Warren (1921) came to the conclusion that the agglutination test does not serve to differentiate strains of gonococci into serological groups. On the other hand, Watabiki (1910), working with only 8 strains and employing the complement-fixation method, divided these 8 strains into subgroups, but his results led him to regard the differences noted

between these subgroups as not distinctive but only comparative. More recently Jötten (1920) investigated 27 strains, and by agglutination divided these into 4 subgroups, of which 5 constituted his group A, 5 group B, 7 group C, and 3 group D, while 7 strains could not be grouped. He also traced a parallelism between agglutination, complement fixation and toxicity for mice, but unfortunately the description of technique in his paper is so meagre that repetition of his experiments presents some difficulty. Hermanies (1921) gives an excellent account, with full description of technique, of the examination of 85 recently isolated strains by the absorption of agglutinins test and finds that 76 of these fall into one of two groups, 41 being designated Type I and 35 Type II, by this author.

Gordon, in a personal communication, January, 1921, informed the writer that on examination by the absorption of agglutinins test of 30 strains isolated in London, 25, owing to their close, though by no means clear-cut, relationship, as disclosed by this test, could be regarded as constituting a fairly well-defined subgroup.

While Torrey, in his earlier work, and Jötten lay stress on the multiplicity of types, Watabiki, Hermanies, Gordon, and Torrey, in his later work, call attention to the greater importance of one or two of these types. All, nevertheless, admit that there are several types, many of which, however, appear to be of less significance owing to their being encountered much less frequently in cases of gonorrhœa.

Technique of agglutination. Only sera having a titre of 1/1,600 or more should be used, and the dilution should be 1/200 to 1/500. Incubation is at 55° C. for 24 hours, and the total volume of fluid in each tube 0·5 c.cm. The suspension of organisms employed is of the strength of 4,000 millions per c.cm.

Technique of absorption of agglutinins. To 2 c.cm. of suspension of the organisms to be examined, standardized to contain 8,000 million cocci per c.cm., there is added sufficient serum to make the concentration equal to ' 16 T.', i.e. if the serum used had a titre of 1/1,600 it is added in a volume of 0·02 c.cm. to the 2 c.cm. of suspension. Groups of 4 or more strains and a control absorption with the homologue should be tested at one time, so that adequate comparison of results is assured, and a tube containing 3 c.cm. of 0·5 per cent. carbolic saline plus sufficient serum to make a concentration equal to ' 16 T.' is set up at the same time in order to determine the degree to which the strains tested are agglutinated in presence of the unabsorbed serum. The mixture of serum and test organisms and the serum diluted in 0·5 per cent. carbolic saline are incubated for 24 hours at 37° C., and the tubes centrifuged to deposit the organisms. The clear supernatant fluid so obtained is then distributed for the second phase of the test, in which the strain under investigation is tested against dilutions of unabsorbed and absorbed serum, the absorbed serum being, as usual, tested against a suspension of its homologue.

Results obtained in applying Absorption of Agglutinins Test to One Hundred Strains of Gonococci.

The following is a résumé of the findings obtained in investigating one hundred strains of the gonococcus by the technique described in the previous subsection :

(*a*) Thirty-seven strains agglutinated to full titre and completely, or almost completely, absorbed the agglutinins from antiserum to the prevalent type of gonococcus.

(*b*) Fifteen strains, although they failed to react to full titre, did completely remove the agglutinins from antiserum to the prevalent type.

(*c*) Twenty-one strains, although they did not agglutinate to full titre, did so markedly absorb antibodies from antiserum to the prevalent type that they may be regarded as prevalent type strains. This subgroup C appears to correspond to the ' intermediate ' strains of Torrey and Buckell (1922).

(*d*) One strain, although it practically failed to agglutinate in presence of the prevalent type antiserum, completely absorbed the agglutinins therefrom. It is worthy of note that Torrey (1922) calls attention to the fact that in a similar series tested out by him he found 54 strains (52 per cent.) to belong to what he terms the regular group—the above figures (*a*) and (*b*), and 20 per cent. to what he terms the intermediate group. The above figure (*c*) is 21 per cent.

There appears, therefore, to be a very close correlation between the findings of the writer and those of Torrey and Buckell (1922) and the unpublished observations of Gordon (1921), the prevalent type observed by Gordon having been proved to be the same as the prevalent type observed by Tulloch (1922). The remaining strains did not lend themselves to serological classification, there being apparently a great variety of serological races represented among them.

It may, therefore, be concluded that the absorption of agglutinins reaction serves to show that there is a predominant type of gonococcus comprising regular and intermediate subgroups which constitutes about 70 per cent. of all strains which are responsible for the causation of gonorrhœa. It is to be noted that the absorption tests quoted were so conducted that serological variation due to the development of colony variants, as observed by Atkin (1925), did not occur.

Complement Fixation.

That the results obtained with complement fixation parallel very closely those obtained with agglutination was early observed by Vannod (1907), and since then results of a similar nature have been published by numerous authors, perhaps the most complete account being that of Torrey and Buckell (1922). These authors state ' the results (of complement fixation) substantiate fully the conclusions which we reached on the

basis of our agglutination experiments that our strains 34 and 42 (representatives of the regular and intermediate subgroups) are both markedly generalized in their affinities'. According to Thomsen and Vollmond (1921), the complement-fixation reaction is to be preferred to the agglutination test, its interpretation being easier. These authors observe that complement fixation alone did not serve to differentiate the gonococci *inter se*, but if they absorbed a serum with the strain to be investigated and subsequently tested the supernatant fluid so obtained by complement-fixation tests conducted quantitatively in the presence of the strain homologous to that used for the preparation of the serum, they found that the strains of the micro-organism which they investigated fell into fairly well-defined subgroups.

By this means, investigating 26 strains, they divided them into 3 subgroups—subgroup A, comprising 14 strains, subgroup B, comprising 5 strains, and subgroup C, comprising 5 strains, with an additional 2 which exhibited a strong affinity for their group C. That is, 14 out of 26 strains constitute a prevalent type in their series.

Serological Classification in relation to Clinical Manifestations.

According to Jötten (1920) certain of the serological types defined by him were especially associated with complications in the clinical course of the infection to which they gave rise, and this possibility was kept in view by Thomsen and Vollmond, but the figures of these investigators and of Jötten are too small to permit of any definite conclusion being drawn from them.

Pathogenic Action.

Pathogenic Action in Laboratory Animals.

So far as laboratory investigation is concerned, but little can be said concerning the pathogenicity of the gonococcus, for its outstanding feature is that its pathogenicity is strictly limited both in nature and, unless under exceptional circumstances, experimentally, to man, intra-urethral inoculation even of apes failing to cause infection (Brück, 1912).

The inoculation of cultures of gonococci into certain animals produces a variety of reactions, but in no instance has there been adduced unequivocal evidence of reproduction of the micro-organism in the tissues of animals other than man, unless these tissues have been rendered specially susceptible by artificial means. Nevertheless, according to De Christmas (1897), one can recover gonococci from the blood of rabbits which have received large doses of cultures intravenously as late as 48 hours after injection.

Notwithstanding the inability of the gonococcus to invade the tissues of animals other than man, its protoplasm, at least after autolysis, appears to be highly toxic to a variety of animal species, as shown by De Christmas (1897). The inoculation of cultures grown for about 10 days at 37° C. in ascites broth into rabbits leads to very definite local and general

disturbance, which, however, is equally well produced by the redissolved alcohol precipitate of filtrates from similar cultures. The general disturbance resulting from intravenous inoculation in the rabbit is characterized by a transient fever, followed by loss of weight, which goes on for about 10 days, after which the animal rapidly recovers. Its injection into closed serous sacs in the rabbit—pleura, anterior ocular chamber, or joints—results in a more or less acute inflammatory disturbance with pus formation, and, if the quantity of material injected is sufficient, this local disturbance is accompanied by a rise in temperature. Similar findings are obtained in goats, mice, and guinea-pigs. In the adult rabbit subcutaneous injection results in a subacute inflammatory condition with some pus formation, and such inflamed areas frequently become the site of secondary infections. In young rabbits the same is seen, but the effects are more pronounced, and sterile abscesses occur at the site of inoculation.

It is specially worthy of note that neither the inoculation of live gonococci nor the instillation of the products of autolysis of the coccus on to any mucous surface of laboratory animals leads to any disturbance whatsoever, although applied to the human urethra, cultures give rise to a definite infection, while the products of growth lead to a sharp and unequivocal inflammatory reaction. There is one mucous membrane which can be apparently infected, viz. the eye of young rabbits, but only when the conjunctiva is previously treated with bile (Kalinin and Fahlberg, 1927). Whether the products of autolysis, which are responsible for certain of the conditions described, can really be regarded as true endotoxin is very doubtful, for they may well be quite different from the toxic elements of the living micro-organism.

Pathogenic Action in Man.

The portals of entry of the gonococcus into the human subject are, practically speaking, two in number, the genito-urinary mucosa, and the conjunctival mucosa, and the lesions produced in both situations are essentially the same.

The earliest stages of infection are characterized by catarrhal inflammation proceeding later to the production of pus. Bumm (1885) was apparently the first to give an adequate description of the process, especially as it occurs in the conjunctiva, and since then there have been numerous publications in which are described the histo-pathology of the condition both in its acute and chronic phases, and more particularly as it affects the mucosa of the genital tract in both sexes. The descriptions given by Finger (1891 and 1894) constitute a very complete account of the process as occurring in the genital organs of the male. More recently, Motz (1903) and Wyeth (1913) have corroborated the observations of Finger and amplified his findings.

The following account of the pathological histology of the condition is based on the descriptions of the authors quoted.

Genital Infection.

It is found that sections prepared on the third day of illness exhibit the presence of a layer of pus on the mucosa, while numerous gonococci are found within and between the pus cells, and but little penetration has occurred, at least in those areas covered by squamous epithelium: desquamation does occur, but not to a great extent. Where, however, the surface of the mucosa consists of cylindrical epithelium, there is evident intercellular penetration of the cocci, and the inflammatory changes are most conspicuous around the mucous glands where gonococci can be seen in large numbers in the perifollicular tissue. The gland epithelium, although considerably distorted from pressure, shows no intracellular invasion with the cocci, and, indeed, exhibits remarkably little disturbance. The gonococci may reach the submucous tissue in areas other than those which are perifollicular, but in all situations it is only the superficial layers of the submucosa which are invaded, and the cellular reaction consists in marked round cell infiltration of the connective tissue, accompanied by dilatation of the capillaries, which are filled with polynuclear cells. The affected areas are naturally œdematous and often the precise situation of the cocci cannot be readily appreciated, but in the main it is found that on the surface many of them are situated within the pus cells and in the connective tissue they are extracellular. The patchy distribution of the process, which is pre-eminently lacunar and perilacunar, is its most striking character.

The condition may undergo almost complete re-solution, but in a large number of cases a subacute or chronic inflammatory condition is liable to remain, with but few gonococci, situated mainly in the glands. It is because of the chronicity of this phase of the process that relapses are liable to occur, and so much difficulty is experienced in offering an opinion concerning the cure of gonorrhœa. With the occurrence of repair, there may be over-proliferation of the columnar epithelial cells, which tend to develop a couch several layers deep, lining the urethra. The columnar cells become polygonal and even squamoid in shape and these squamoid cells may occasionally become keratinized.

The repair process in the submucosa is characterized by the formation of fibre tissue, which, by its contraction, causes distortion of the parts, and, in the male, may lead to fibrotic obliteration of the follicles of Littré and the lacunæ of Morgagni—a process of natural cure. If the process has been severe and has occurred over a fairly extended area of the urethral mucosa, the contraction of the fibre tissue brings about urethral stricture.

The inflammatory process passes both along the surface of the mucosa and in the submucous tissue, and, when the latter is infected, dissemination by the lymphatics and even by the blood-stream occurs and may give rise to the more remote manifestations of gonococcal infection. Spread by surface continuity is responsible for posterior urethritis, which, because of the anatomical relationships of the posterior urethra in the

male, may be regarded as of special significance. In the membranous portion of the urethra the presence of the sphincter has an important influence on the pathology of gonorrhœa, as the contraction of that muscle is liable to lead to fissuring of the inflamed urethra, and the fissures, healing by fibrosis, are prone to bring about stricture. Involvement of the prostate by catarrhal or purulent inflammation of its acini and similar infections with corresponding changes in the ejaculatory ducts, vesiculæ seminales, and Cowper's glands are also liable to occur.

The disease may extend to the epididymis and, according to Luys (1922), does so in about one in every four cases. The demonstration of the gonococcus in the pus of epididymitis is not easy, as the organisms are usually present only in small numbers, especially when the process has become purulent, but Baermann (1903) succeeded in obtaining cultures in a large series of cases. It is a debatable point whether the micro-organism reaches the epididymis via the lymphatics or by direct continuity of surface along the vasa deferentia, the former view being held by Nobl (1901) and the latter by Ullmann (1901). The changes which occur in the epididymis are similar in nature to those which occur in the urethra, and the principal danger is obliteration of the vasa efferentia by the process of fibrosis when healing takes place.

A further extension *per continuitatem* is to the bladder, in which organ the infection, according to Finger (1905), results in proliferation of the submucous tissue ; the vessels there are congested and contain numerous polynuclear cells, within many of which gonococci can be demonstrated. Accompanying this change the epithelium of the organ undergoes desquamation.

Further extension of infection in the urinary tract may occur, leading to pyelitis and pyelo-nephritis, but the precise mechanism of such extension is somewhat doubtful ; there are three possibilities to be considered—extension by continuity, extension by lymphatics, and infection via the blood-stream.

Ano-rectal infections also occur by continuity in the female, and the pathological process in this situation is similar to that which takes place in the urinary tract. Indeed, in females, extension of infection to the ano-rectal mucosa appears to be relatively common, occurring in 31·5 per cent. of (female) cases according to Baer (1896), and according to Huber (1898) about 18 per cent. Extension of the condition to the seminal vesicles is important, as, owing to their vascularity, generalization of infection by the blood is liable to occur when they are involved.

In the female urogenital tract the nature of the inflammatory process is the same as that which has already been described as occurring in the male organs, and the differences in the pathology of the condition as affecting the two sexes depend for the most part upon differences in the anatomical arrangements of the parts. Gonorrhœal urethritis in the female is the exact counterpart of the corresponding condition in the male, and the mucous glands of the urethra as well as those which are situated

around the urinary meatus play the same part in maintaining the infectivity in the female as do the glands of Littré in the male.

Vaginal infection deserves special consideration for two reasons. In the first place there appears to be but little doubt that in children this mucous surface is much more susceptible to infection than in the adult. This observation has, on the one hand, important medico-legal bearings, and, on the other, serves to explain the occurrence of indirect infection in female children through the medium of soiled bed-clothing and other inanimate objects—a type of infection which is rarely proved in the adult. Some remarkable institutional outbreaks of gonorrhœa traceable to the use of common towels, night-clothes, bed-linen, sponges, &c.—(Hamilton, 1908 and Dowd, 1921)—have been described.

Secondly, the termination of acute vaginitis is in no way an indication of cure, for, in the majority of cases, infection extends to the cervix uteri, the glands of which may remain infected for a prolonged period. The statistics of cervical involvement show that extension to this area occurs in about 60 per cent. of cases according to Heiman (1895, 1896 and 1898). The glands of Bartholini may be involved in a fashion similar to those of the urethra, and when the duct is occluded a Bartholinian abscess forms. The abscess may discharge through a fistula or sinus which continues to exude infective material for a prolonged period.

Extension by continuity to the body of the uterus is fortunately of less frequent occurrence and usually results in chronic endometritis, which, in turn, may lead to salpingitis and oophoritis. Extension to these organs is estimated by Marschalko (1900) to occur in about 40 per cent. of cases of chronic gonorrhœa in females. It is worthy of note that the examination of the contents of the tubes in cases of chronic gonococcal salpingitis may fail to reveal the presence of the causal organism.

The most grave danger in the female is involvement of the peritoneum, either by extension of infection through the walls of the Fallopian tubes, or along the surface and through their fimbriated extremities. In most instances, the process, when it does extend to the peritoneum, is shut off, so that the condition is usually localized and chronic, and only occasionally are cases of acute peritonitis encountered. In some of the acute cases the causal organisms have been demonstrated both microscopically and by culture. Foulerton (1905) described such a case with full notes.

Gonococcal Conjunctivitis.

The pathological changes in gonococcal conjunctivitis are of the same nature as those which occur in gonococcal infection elsewhere, and were fully described by Bumm (1885). The condition is of special significance on account of the marked susceptibility of the mucous surface of the conjunctiva to infection in new-born children during birth from the mucosa of the genital tract of the mother. The disease is easily prevented by instilling into the conjunctiva of the new-born a few drops of a suitable antiseptic. Gonococcal conjunctivitis is one of the gravest social results

of gonorrhœa on account of the danger of corneal ulceration leading to perforation of the cornea and the development of anterior polar cataract, and even panophthalmitis may result in this condition. It is stated that occasionally the infection may be already established before birth in those cases where premature rupture of the membranes has allowed infective material to gain entrance to the amnion and labour has been sufficiently prolonged to permit of the infection being established in the conjunctiva of the child.

Generalized Gonococcal Infections.

In a small percentage of cases of gonorrhœa generalized infection occurs, and this may be manifested in a variety of ways. It is more liable to occur in the male than in the female and in those cases in which gonorrhœa has become chronic and has involved the seminal vesicles, epididymis, or the prostate ; very occasionally it may even be encountered in the acute phases of the illness. Irons (1909) gives a full clinical and laboratory account of six representative cases of blood infection with the gonococcus in which the diagnosis was verified in each instance by blood culture. The septicæmia is practically always accompanied by the development of metastatic lesions. The commonest of these is arthritis, with which may be grouped synovitis, and the most serious is endocarditis. The incidence of gonorrhœal arthritis is estimated by Neisser and Scholtz (1903) to be a little under 1 per cent. of all cases of gonorrhœa.

In arthritis, when the inflammatory condition is acute, there is not much difficulty experienced in cultivating the micro-organism from the joint exudate, but in the majority of cases the acute manifestations have usually passed before bacteriological examination is made and then the micro-organisms are few in number and may be found only in the tissues of the inflamed joint capsule and synovial membranes, and in such circumstances the cultivation of the micro-organism from the lesions presents great difficulty.

A review of a large number of communications dealing with gonococcal arthritis was published by Chiari (1914), and the reader is referred thereto for further information on the subject.

Gonococcal osteo-periostitis is sometimes associated with arthritis, but in most instances the condition is really only an aspect of the fibrosis which is associated with the healing of the joint lesions. There are, however, authentic instances of true gonococcal periostitis and osteomyelitis, but these are of very infrequent occurrence. Finger, Ghon and Schlagenhaufer (1894, 1895) described a case of purulent periostitis and proved the invading organism to be the gonococcus on complete cultural examination, while Watts (1911) gives an account of a case of osteomyelitis and quotes three further cases from the literature.

Gonococcal endocarditis is worthy of special note on account of its danger to life and health. This complication is more frequent in men than in women, and its onset may occur at any period during the course

of an acute or chronic infection. The lesions produced are those of an ulcerative endocarditis, resembling the type found in subacute infective endocarditis, but in some instances the condition of the cardiac valves is akin to that observed in so-called simple endocarditis. Some of the authentic cases of gonococcal endocarditis published in the literature are of special interest, as, e.g., that described by Ghon and Schlagenhaufer (1898), in which a culture prepared from the inflamed valve gave rise on injection into the urethra of a volunteer, to a typical attack of gonorrhœa. It is to be emphasized that, in many of the cases of presumed gonococcal endocarditis described, the evidence that the inflammatory condition of the heart was due to infection with gonococci is incomplete, in that cultures were not made; nevertheless there is a very extensive literature in which such proof is adduced. In some cases the infection spreads from the valve to the myocardium, giving rise to a localized myocarditis.

Other serous surfaces—apart from the peritoneum—are but seldom infected with gonococci, but cases of gonococcal pleurisy and pericarditis have been reported. The authenticity of these conditions has, however, been too infrequently proved by cultural methods. Among the cases that are definitely proved is one described by Géraud and Johnston-Lavis (1911–12), in which the nature of the invading organism in an empyema was indubitably the gonococcus. These authors give a full bibliography, including cases described by Mazza in 1894, and in 1899 by Cardile, in which also evidence of gonococcal infection of the pleura appears to be unequivocal.

The meninges are occasionally invaded as an incident in generalized gonococcal infection, but the accounts of many of the published cases of gonococcal meningitis, so called, are incomplete in that the laboratory data are inadequate. There have, however, been several well-authenticated cases of this infection, and a full account of the subject is given by Lindenfeld (1922). Involvement of the brain substance itself appears to be very unusual, but Larkin and Jelliffe (1913) described a case of encephalitis in which the evidence that the invading micro-organism was the gonococcus was more than suggestive, although perhaps not unequivocal.

Eye Infections other than Conjunctivitis.

There appears to be a positive correlation between affections of the uveal tract and gonorrhœa, but definite evidence that iridocyclitis and choroiditis are really due to metastatic invasion of the uvea with gonococci, is lacking. This is to be explained partly at least by the fact that bacteriological examination of the part is difficult, and there is, therefore, a natural tendency to explain such eye conditions as purely ' toxic ' manifestations of the gonorrhœal process, but such explanation only serves to emphasize our ignorance of what actually is the nature of the correlation between uveitis and gonorrhœa, for occasional cases of definite

metastatic infection of the eye, proved by bacteriological investigation and occurring in generalized gonococcal infection, have been described in the literature.

Mechanism of Pathogenicity.

The mechanism of the pathogenicity of the gonococcus has not received much attention from investigators, and, indeed, largely because of the fact that this micro-organism is not susceptible of investigation by animal experiment, we remain ignorant both of its mechanism of invasion and its mechanism of disease causation. In the case of a small number of the pathogenic protophyta, notably those whose pathogenicity depends upon their elaborating exotoxins, the term virulence may, within limits, be regarded as synonymous with toxigenic capacity, but in the case of a large number of disease-producing micro-organisms virulence is really an expression of invasive capacity, and only in very few instances, notably in the case of the gas gangrene bacilli, is the mechanism of the invasive process susceptible of experimental investigation. We have no adequate method of assessing the virulence of most micro-organisms either in terms of their invasive capacity or of their toxigenic properties, and this is particularly true of the gonococcus.

Although the evidence that the gonococcus depends for its infective power upon the elaboration of special aggressive properties is but slight, there is more than a suggestion that such properties do play some part in determining its pathogenicity. Thus, according to Pinto (1904), serial passage in young rabbits will greatly enhance the infective capacity of the gonococcus for rabbits. Very small doses—0·00005 c.cm. per kgm. of such passage strains—sometimes give rise to septicæmia in these animals. One is driven to conclude from the smallness of the effective doses, from evidence of hæmorrhagic septicæmia in the animals, and from the fact that cultures could be obtained from their blood, that the invasive or aggressive properties, as distinct from the toxicity of the coccus, at least for the rabbit, were modified in Pinto's experiment, and it is, therefore, probable that variation of this quality occurs naturally, so influencing the infective capacity of the gonococcus for man. Pinto's findings have been partially corroborated by Bruckner and Cristéanu (1906), and bear a close resemblance to Murray's (1923–4) observations on the infectivity of the meningococcus for laboratory animals. It is of interest to note that Pinto lays stress on the hæmorrhagic character of the septicæmia, and suggests that the gonococcus is endowed with hæmolytic qualities.

The question of correlating what has been referred to above as 'invasive capacity' with the aggressive qualities exhibited by extracts and autolysates of the gonococcus as demonstrated by Wollstein (1907), by Bruschettini and Ansaldo (1907), and by Warden (1915), presents some difficulty, but the balance of evidence is that although such products are definitely toxic, and although their presence may enhance the toxicity

of living suspensions of the organism, they do not appear markedly to increase the *infectivity* of the micro-organism for laboratory animals. There is, therefore, another aspect to the mechanism of pathogenicity of the gonococcus, viz. its toxicity. In the first place it is to be emphasized that no investigator has so far succeeded in demonstrating the production *in vitro* of a true exotoxin by the organism, although De Christmas (1900) interprets certain of his findings as indicating the production of a substance akin to exotoxin in culture. We are, therefore, forced to conclude that the pathogenicity of the gonococcus is partly at least dependent upon toxic qualities contained within, or liberated by lysis of the substance of, the coccus itself. Much work has been published dealing with the toxic and lethal action of dead cultures and of autolysates of gonococci, but it is very doubtful indeed, notwithstanding the very ingenious methods which have been employed, whether anything really approximating that very elusive substance, the true *endotoxin* of this micro-organism has yet been obtained.

None the less, filtrates and autolysates and cultures of the gonococcus exhibit a considerable degree of toxicity ; De Christmas (1897 and 1900) gives a very full account of the effects produced on inoculating various animals by different routes with old cultures. Guinea-pigs, mice, rabbits and goats were all susceptible, but the ' lethal properties of the so-called toxin ' were not of a high order, at least 1 to 2 c.cm. being required to kill a guinea-pig by subcutaneous or intraperitoneal inoculation. The most striking effects of the toxin described by De Christmas were obtained by intra-ocular (anterior chamber) and intracerebral inoculation ; the former leads to an acute inflammation with hypopyon, and the latter may be lethal for a guinea-pig of 250 gm. weight even in so small a dose as ·001 c.cm. of a 30 days' fluid culture. The fact that the maximum toxicity of such products is attained only after prolonged incubation, strongly suggests that the toxic quality of the material is due to autolysis of the cocci. The introduction of such ' toxins ' into the human urethra leads, within an hour or two, to a marked inflammatory condition of the urethral mucosa with the production of pus ; this inflammatory reaction is of short duration.

The present position of our knowledge concerning the mechanism of the pathogenicity of the gonococcus may be summarized thus :

1. There is some evidence that the gonococcus is endowed with a mechanism which determines its invasive capacity, but the nature of this mechanism remains obscure, for, so far, investigators have failed by *in vitro* methods to demonstrate the production of aggressive substances during the early phases of growth.
2. The products of autolysis and suspensions of dead gonococci are toxic in the sense that large doses cause illness, and even death of laboratory animals, when administered *sub cutem*, into the serous sacs, or intravenously, while small doses of filtrates of old cultures administered intracerebrally have the same effect.

3. As in the case of other micro-organisms whose pathogenicity is alleged to depend upon their 'endotoxins' we have no evidence that these toxic filtrates really represent true endotoxins.

We have, therefore, frankly to admit ignorance of the precise nature both of the invasive mechanism and of the disease-producing mechanism of the gonococcus.

Viability.

It is fortunate that the viability of the gonococcus is of a low order, for were this not so, the prevalence of gonococcal infection would be even greater than it is, and we should be called upon to deal not with small but with large numbers of cases of the disease acquired innocently. The various deleterious influences which may be exerted upon the gonococcus either naturally or artificially can be considered under the following headings:

Influence of Drying.

The gonococcus is peculiarly susceptible to desiccation, and, even in morbid exudates, it very soon dies unless conditions are such as to prevent loss of moisture from the material. It is difficult to determine accurately the viability outside the body, because cultural methods, even the best, are really inadequate for this purpose. Therefore, it is by no means easy experimentally to investigate the influence which drying exerts upon the viability. That the organism may survive in fluid pus at room temperature for a period of at least 24 hours is well known, and recently in this laboratory (Bacteriological Laboratory, University College, Dundee, University of St. Andrews) we have successfully cultivated the gonococcus from a joint fluid that had been retained at room temperature for this period.

Probably, in common with other micro-organisms, the process of slow desiccation exerts an even more deleterious influence upon the gonococcus than does very rapid dessication, but even when dried *in vacuo* over sulphuric acid, it is unusual to find that the cocci have survived (Cruickshank, 1923), although the technique of drying employed by that author permits of a remarkably prolonged maintenance of the life of nearly all other micro-organisms, including the meningococcus. According to Finger, Ghon and Schlagenhaufer (1894), the organisms remain alive in discharges only so long as these are moist; Neisser and Scholtz (1903) agree with this, stating that the organism died rapidly in pus when dried, but could survive in moist pus up to 24 hours. It is probable that there is considerable variation in the susceptibility to drying exhibited by different strains, but definite evidence of this is difficult to adduce.

Influence of Temperature.

The influence which exposure to various temperatures exerts upon the gonococcus is also difficult to assess, for, when exudates are investigated

under conditions resembling those in which they may carry infection from one individual to another, the problem is complicated by drying of the material, while in cultures there is a range of temperature over which the autolysis of the organism may exert disturbing influences.

Mammalian temperature and 'room' temperature. Provided that they are kept moist, cultures of gonococci may remain alive at 37° C. for prolonged periods. Thus Torrey and Buckell (1922) quote an instance in which an old stock strain survived in semi-solid agar of reaction pH 6·4 at 37° C. for over a year, and it is by no means unusual to maintain even recently isolated cultures for over a month in this medium at mammalian temperature. At room temperature under the same conditions the period of viability is very much shorter, but the writer has observed survival for at least 8 days.

Temperatures under freezing-point. If the micro-organism is cultivated at 37° C. and then exposed to low temperatures, the viability of certain units of the cultures is considerable, as was shown by Lumière and Chevrotier (1914) who obtained subcultures after exposure of growths to a temperature of minus 20° C. for 10 days. This result was partially verified by Thomson (1923) who found that young (24-hour) cultures on his medium could be placed in the ice-chest for 12 days, and after such treatment still gave subcultures. These were, however, slow in growing, and the quality of the growth described by Thomson strongly suggests that only a few of the micro-organisms remained alive in his experiments. The writer has on several occasions placed 24-hour cultures of recently isolated gonococci grown on the medium of Torrey and Buckell in a thermostat at minus 10° C., and found that the period of survival in such conditions was very variable indeed, viz. from 24 hours to 10 days, and the majority of strains did not even survive the shorter period of exposure to these conditions.

According to Lumière and Chevrotier (1914), gonococci can be sub-cultured from growths after these have been exposed to minus 195° C. for 24 hours. This indicates that if the organism is kept moist and is not exposed to definitely deleterious conditions, it may survive conditions of extreme rigour. Of the deleterious conditions alluded to, suspending the cultures in 0·9 per cent. NaCl may be given as an instance. This is definitely toxic to the gonococcus, as it is to other micro-organisms, e.g. *B. coli* and the meningococcus (Shearer, 1919).

Temperatures above average mammalian temperature. The gonococcus is extremely susceptible to temperatures over that of 37·5° C., and attention has already been directed to the importance of this in the discussion of its isolation and cultivation. In this connection it must be appreciated that although a slight elevation in temperature—1 or 2° C.—may interfere with the growth of cultures and may possibly lead to their death by promoting autolysis, it does not follow that in its natural habitat gonococcus is necessarily killed by a similar elevation of temperature, such as might occur in acute febrile conditions. This warning is given

as there has been a tendency to base therapeutic methods in gonorrhœa on the production of febrile reactions by the parenteral injection of foreign proteins such as milk. It is now generally accepted that, in cultures, the gonococcus is killed in a few hours if exposed to 40 to 41° C., a fact to which attention was drawn by several observers including Steinschneider and Schäffer (1895), but that in the tissues it will remain alive when exposed to febrile conditions even in excess of 40° C. Information concerning its thermal death-point in lesions is furnished by Boerner and Santos (1914) and Santos (1913–14), who found that gonococci in the urethra were killed by exposure to 102·2° F. for 10 hours, while 107° F. was definitely lethal in about 1 hour, these temperatures being attained by diathermy.

Influence of Exposure to Light.

The gonococcus is, as is almost to be expected, susceptible to the shorter light wave-lengths and rapidly dies when exposed to sunlight or to any source of ultra-violet rays artificially produced.

Influence of Chemical Bactericides.

It will be readily understood that the investigation of the influence of bactericides and antiseptics upon the gonococcus presents technical difficulties of no mean order. The results of tests conducted *in vitro* must be critically scrutinized, for it is difficult enough in any circumstances to cultivate the gonococcus, and, when the effect of chemical substances form the subject of study, meticulous care must be exercised to ensure that the death of the test organism has really been due to its exposure to the substance tested, and not to some minor error of technique inherent in the method employed for conducting the tests. It must be emphasized that the results of *in vitro* tests bear no, or only a very remote and indirect, relationship to the bactericidal or antiseptic value of reagents employed therapeutically, and for this reason *in vitro* study of bactericides and antiseptics in their relationship to the gonococcus may be regarded almost as a work of supererogation.

Steinschneider and Schäffer (1894) called attention to the marked bactericidal activity of silver compounds upon the gonococcus, while Finger, Ghon and Schlagenhaufer (1894) showed that in serum-agar culture it was not so remarkably susceptible to chemical bactericides as might be expected. A full account of investigations dealing with these reagents is given by Swartz and Davis (1921), their general conclusions being that the bactericidal activity of the substances tested—argyrol, protargol, silvol and cargentos—was of a low order. Of the aniline dyes, acraflavine has received most attention, and at least *in vitro* possesses a high degree of activity, killing the gonococcus in 1 minute when diluted 1 in 70,000 (Thomson, 1923).

'Mercurochrome 220' has also been much studied recently, and according to Swartz and Davis (1921) is lethal in 20 minutes when diluted 1 in 16,000.

Natural Resistance in Animals.

Attempts have been made to infect various animal species with gonococci, but the results have been negative in the sense that reproduction of the cocci in the inocula does not occur, and the pathological changes which follow such inoculations are produced as readily by dead as by living cultures. So far as inoculation of mucous membranes is concerned the great majority of investigators from Neisser (1879) and Leistikow (1882) to Joseph Koch and Sieskind (1912) obtained solely negative results. Brück (1912) states categorically that he failed to produce gonorrhœa in the anthropoid apes by intra-urethral inoculation of gonococci. The exceptions to these negative findings are few, but Colombini (1898) claims to have inoculated successfully the genital mucosa in both rabbits and dogs; while Legrain and Legay (1891) and Heller (1896) state that they have produced purulent conjunctivitis, the former in guinea-pigs and the latter in young rabbits, by inoculation with gonococcal exudates or cultures; but it is probable, as maintained by Morax (1894), that results such as these are really due to inflammatory disturbances of purely toxic origin, and are as well produced by dead as by living cultures.

The animals which appear to be most susceptible to intraperitoneal inoculation are white mice, and, according to Wertheim (1891), gonococci can be recovered from the peritoneal exudate of these sometimes as late as five days after inoculation. Wertheim's results have been corroborated by several authors, and, in a certain number of instances, the gonococcus has been recovered from the heart blood. The description by Nicolaysen (1897) of the findings in his experimental animals is recalled by those of Murray (1923–4) with meningococci. Nevertheless, Nicolaysen did not conclude, notwithstanding the occurrence of positive blood cultures in his series of experiments, that active reproduction of the inoculated cocci had taken place. Positive blood cultures in experimental animals are described by various authors—Morax (1894), Scholtz (1899) and Jundell (1900). Wildbolz (1902) also observed the occurrence of generalized infections in guinea-pigs after inoculation of old cultures of the micro-organism, which, in the main, consisted of involution forms, and he concluded from the appearance of the micro-organisms in the blood that true reproduction and presumably invasion of the tissues had occurred. Injection has been made into the other serous cavities of experimental animals, pleura, pericardium, joints and meninges, but in the main the results have failed to indicate reproduction of the micro-organisms and true invasion of the tissues. The same appears to be true of intravenous injections, although Pompéani (1898) recovered the organism from the blood of experimental animals up to 48 hours after intravenous inoculation of live cultures.

Inoculation of the anterior chamber of the eye in rabbits is followed by marked inflammatory disturbance and hypopyon, but this condition does

not appear to be due to infection, for the majority of investigators find that the pus is sterile (Brück, 1912; Mezincescu and Holban, 1919). It may, therefore, be concluded that the gonococcus does not naturally infect animals other than man, although it is possible by the use of special methods, with large doses of culture, to produce in a limited number of animals, conditions in which gonococci can for a limited period be recovered from the blood-stream. It appears that the lethal action of gonococci on experimental animals differs from strain to strain, and even in the same strain at different times and under different conditions of culture. Such variation probably depends upon several factors, such as toxicity, invasive capacity and adaptation to new environment, but which of these is of prime importance in determining increase in pathogenicity under any given experimental conditions is not known.

It is apparently true that prolonged culture markedly reduces the pathogenicity of the organism, as shown by Wollstein (1907) and by Torrey (1908). Increase in pathogenicity has been observed by Pinto (1904) to follow serial passage through rabbits, using intraperitoneal inoculation, and by Bruckner and Cristéanu (1906) by a similar procedure in rabbits and cats.

Resistance in Man.

Natural Resistance depending upon Histological Structure.

The mucous membranes covered with stratified epithelium are much less susceptible to gonococcal invasion than are those covered with cylindrical epithelium; with the greater tendency to hardening of the surface layers of the former, the more decided is its insusceptibility to infection. It is this factor which probably plays an important part in determining the much greater susceptibility of the vulva and vagina in children than in adults. The importance of local conditions as predisposing factors to infection in gonorrhœa is also emphasized by Brück (1912), who found that excoriation or maceration of an epithelial surface greatly enhances its susceptibility.

Natural Resistance depending upon Constitutional Factors.

While anatomical relationships and histological structure probably play a paramount part in determining the susceptibility, or its converse, of an individual to gonococcal infection, the possibility that some people are constitutionally more susceptible than others cannot be ignored. Indeed, by analogy with other infections it would be surprising if such differences did not exist, but we have no means at our disposal for determining the conditions upon which they depend. The only indication of such constitutional variation obtainable is from clinical observation of cases of gonorrhœa with metastatic infections, but information from this source is difficult to interpret, as metastatic infection itself may depend upon anatomical and histological factors at the site of the primary infection

and possibly also upon the infection being due to an especially invasive strain of the micro-organism. Jadassohn (1898) quotes instances, which may have some bearing upon this problem, of individuals who have acquired gonorrhœa more than once and have on each occasion developed metastatic lesions. He cites the case of two brothers, both of whom acquired gonorrhœa from different sources, and developed metastatic infections. Familial susceptibility to gonococcal invasion is a possible explanation of the facts of this case, but is obviously not the only one compatible with them.

Naturally acquired resistance through contact with subinfective doses of the virus, a mechanism which appears largely to determine the limitation of propagation of other communicable disease, does not, from the very nature of the malady, play an important part in determining immunity to gonorrhœa.

Practical Diagnosis.

The practical methods of diagnosis of gonorrhœa by laboratory methods naturally fall into two categories :

(*a*) Direct demonstration of the gonococcus in morbid exudates.
(*b*) Indirect methods of diagnosis—provocative tests, skin reactions, and serological tests.

Direct Methods.

Direct Microscopical Examination.

Acute and subacute infections. When dealing with an acute case of gonorrhœa, either in the male or female, but little difficulty is experienced in arriving at a definite diagnosis by microscopical examination of the discharge. The methods used for staining films of the discharges, and the appearances observed have already been dealt with (see p. 243).

Chronic gonorrhœa in the male. Although the diagnosis of the acute phases of gonococcal infection is so simple it cannot adequately be emphasized that the diagnosis in chronic cases is often extremely difficult, and, as such cases are of paramount importance in maintaining the disease in the community and of equal importance in forensic medicine, the diagnosis of chronic gonorrhœa is worthy of special consideration.

As the infecting micro-organism may persist for very lengthy periods in the various glands of the genital tract, special care should be exercised in the examination of the secretions from these, and also of centrifugalized urinary deposit. The technique of collecting these materials is not discussed, as it properly belongs to the sphere of clinical surgery.

Gonorrhœa in the female. The fact which deserves special emphasis is, that, although the examination of vulvar and vaginal discharge is of great diagnostic significance in children, this is not so in adults. A negative finding in the case of women, when such discharges are examined, gives no information whatsoever for refuting a diagnosis of the disease.

On the whole, the diagnosis of gonorrhœa in the adult female is considerably more difficult than in the male, and examination should be made of :

1. The urethra.
2. The peri-urethral glands, which correspond to the follicles of Littré in the male.
3. Cervix uteri.
4. The glands of Bartholini.

The films prepared from these sources should be dealt with exactly as in the examination of a case of chronic gonorrhœa in the male, and the interpretation of the findings should be as conservative as in that condition, for, notwithstanding most conscientious examination of material from all these sources a negative result is of little significance. It is worthy of note, in making examination of the cervix, that any condition in which the cervical mucous glands are active, greatly assists in determining the presence of gonococci in that situation, and, for this reason, positive results may occasionally be obtained in cases that appear to be free from gonococci if the investigation be made during the early part of a menstrual period.

Secondary Infections in Gonorrhœa.

In all cases of gonorrhœa there is a tendency to secondary infection, a tendency which is enhanced by mischievously enthusiastic treatment. Therefore, when the disease has become chronic, it is not unusual to find a vary varied flora in the exudate. These organisms of secondary infection are very numerous in some cases, and may render the examination of material difficult for two reasons. In the first place, certain of the Gram-positive cocci of secondary infection may, especially when phagocytosed, lose their Gram-positive qualities, and are then liable to be mistaken for intracellular Gram-negative cocci. Secondly, it not infrequently happens that, owing to the wealth of these secondarily infecting organisms, the eye is liable to be deceived, so that in the presence of an excessive secondary infection a negative report is not of the same significance as when secondary infection is less pronounced.

It is difficult to determine the significance of secondary infections in the pathology of gonorrhœa, but it may be said that their presence in secretions is evidence of a pathological condition, at least in the male, for the normal genital tract does not contain micro-organisms in large numbers unless in the immediate vicinity of the meatus. It is not improbable, too, that such secondary infection plays some part in maintaining a subacute or chronic inflammation in the urethra and its diverticula. In the female the findings are a little more difficult to interpret, for the flora of the female genitals is more varied than in the male, and it is not easy clearly to differentiate between the flora of normal, or almost normal, and pathological conditions, especially during pregnancy. With this reservation, however, secondary infection in the female is probably of the same significance as secondary infection in the male.

Gram-negative Diplococci other than the Gonococcus in Exudates from the Uro-Genital Tract.

Although the demonstration of Gram-negative diplococci in discharges from the genito-urinary tract is, under all circumstances, very suggestive of gonococcal infection, and, when the cocci are typical in shape, size, staining properties and arrangement, constitutes almost unequivocal evidence of gonococcal infection, it must be appreciated that other Gram-negative cocci may sometimes be obtained in such material. Steinschneider (1889) showed that extracellular Gram-negative cocci which were not gonococci, could be demonstrated in about 5 per cent. of male urethræ, and similar observations were made by Hogge (1893), who observed that these ' pseudo-gonococci ' were rather more difficult to decolorize on applying Gram's method than were true gonococci. A very complete account of the occurrence of Gram-negative diplococci other than the gonococcus in exudates from the female genitals is given by Gurd (1908), who, in the course of routine examinations of material derived for the most part from suspected cases of gonorrhœa, 113 in number, observed such ' pseudo-gonococci ' in 7 instances. Cultural investigation proved 4 of these to be *Micrococcus catarrhalis*, 2 of them *Micrococcus pharyngis*, and 1 *Diplococcus magnus* of Rosenthal (1899).

Diagnosis by Culture of Exudates.

Of recent years, the establishment of venereal clinics by the Ministry of Health has led to surveys of the value of diagnosis by examination of stained films. In actual practice, especially in the female, this method is not only tedious but subject to more error than is generally recognized. For this reason in some laboratories (for instance at the London Hospital) the film method has been abandoned in favour of cultures. The medium (horse serum agar ; vide Vol. IX) is tubed and supplied to the clinic, where officers make the cultures. The tubes are incubated within an hour and examined in the laboratory in 48 hours. In the great majority of cases after a little practice the positive tubes can be recognized by mere inspection with a hand lens. The opinion thus formed may be confirmed by microscopic examination of films from the colonies. In a sample series of 200 examinations, no case was found positive by the film method and negative by culture (though some were dubious by the former), while nearly 10 per cent. were positive by culture and negative or dubious by film.

INDIRECT METHODS OF DIAGNOSIS.

The indirect methods of diagnosis employed in the investigation of gonorrhœa are: provocative methods, specific and non-specific, dermal reactions, and serological tests *in vitro*.

Provocative Methods.

The rationale of the provocative methods is to cause a temporary exacerbation of the inflammatory process so that the exudate therefrom

is increased, thus making easier the demonstration of the gonococcus in discharges from chronic cases. These methods have their value, but must be used with discretion. As a means of actually diagnosing gonorrhœa, provocative reactions have only a limited utility, their value lying more in their application as a test of cure where latent gonococcal infection is suspected.

Non-specific provocative methods. The time-honoured procedure consists in the patient consuming a liberal supply of spiritous liquor with resultant urethral irritation, and a so-called relapse of gonorrhœa. Needless to say, such a method of diagnosis or test of cure is not to be advised.

The topical application of irritants has the same effect, and, if properly carried out, is of considerable value. The irritant usually employed is silver nitrate, its effect being to cause increased flow of fluid, carrying with it to the surface the more deeply situated cocci of latent infection. The cocci can then be demonstrated by microscopical examination of the discharge. This test is of use in the investigation of chronic gonorrhœa in the female, where diagnosis is always difficult, for, in some degree it combines diagnosis with treatment.

Specific provocative methods. The commonly used specific provocative method consists in the subcutaneous inoculation of gonococcus vaccine, which, in the subject of latent gonorrhœal infection, gives rise to reactions resembling to some extent those obtained by inoculation with tuberculin into the victims of tuberculosis. Like the tuberculin test and other similar tests, which seem to depend upon a so-called allergic condition of the tissues of the individual upon whom the test is made, the reaction produced must be interpreted with care. The response may be general, focal and local, but which of these is to be chosen as the index of a positive reaction is doubtful, as also is the optimum dose of vaccine required to produce an indubitable reaction in the majority of positive cases, without at the same time giving positive reactions in cases which are negative. As the provocative effect of the vaccine varies from one individual to another and also from one batch of vaccine to another, as observed by Brandweiner and Hoch (1913), it is difficult definitely to state the optimum dose. According to Irons (1908) a dose of 500 million killed cocci does not produce reaction in normal persons, but does give definite reactions, both focal and general, in gonorrhœal cases. With the statement of Irons must be contrasted that of Stockman (1911), that a dose of 150 millions may produce a general reaction in absence of gonorrhœa. Stockman's findings indicate that the general reaction at least, which follows the injection of a provocative dose of gonococcal vaccine, does not constitute a reliable index of gonococcal infection. The same appears to be true of the local reaction at the site of injection of the provocative dose, such local reactions being liable to occur in non-gonorrhœal subjects.

The focal reaction, then, appears to be the most reliable of the three. According to Frohnstein (1913), out of 30 cases of chronic gonorrhœa in the discharge from which gonococci could not be demonstrated prior to

provocative vaccination, 14 exhibited a definite increase in urethral discharge in which gonococci *could* be demonstrated after the application of that procedure. It may be stated that as a diagnostic method and as a test of cure, provocative vaccination is of some value, but the test is of limited application, and the results obtained must be interpreted both critically and conservatively.

The injection, into the urethra of a normal person, of old fluid cultures or suspensions of the gonococcus, autolysed or killed by exposure to heat, may give rise to inflammatory disturbance. It would seem reasonable to expect that if a dose of such material, insufficient to produce this reaction in a normal person, were injected into the urethra of one who is the subject of latent gonorrhœa, a fairly sharp reaction would occur. Tests of this nature might be regarded as specific topical provocative reactions. Although, so far, such topical provocative reactions of presumably a specific nature have not been extensively used for clinical purposes—diagnosis or test of cure—their employment has been recently suggested by Lambkin, Dimond and Robertson (1927), who use cultures grown on specially prepared agar for this purpose. This method is still on trial and its value has not been assessed.

Dermal Reactions.

The possibility of employing a cutaneous reaction, similar to the tuberculin test, in the investigation of gonorrhœa, was suggested by the observation of Brück (1909), of Reiter (1911), and of others, that when gonococcal vaccine was used therapeutically there might occur marked skin reactions at the site of its inoculation. As a result of these observations, glycerinated extract of autolysed cultures of gonococci administered intradermally came to be used as a method of diagnosis. Irons (1912) employed such glycerinated extracts, and, conducting his test by a technique similar to that of the Von Pirquet tuberculin reaction, obtained fairly definite cutaneous reactions consisting of a zone of hyperæmia from 5 to 10 mm. in diameter, and developing in 12 to 24 hours subsequent to the administration of the test material. Irons states that in normal persons there may be some reaction, but this does not usually extend beyond a diameter of 2 to 3 mm., although in a few individuals there is some difficulty in interpreting the findings owing to the occurrence of marked reactions where previous gonococcal infection can almost certainly be excluded.

It is not necessary to employ glycerinated extracts, for, according to London (1912), the test can be carried out with ordinary gonococcal vaccine standardized to contain 50 to 100 million gonococci per c.cm., a few drops of this being injected intradermally as in conducting the Schick test. London claims that by this method only negative reactions are obtained in normal persons, even when vaccines containing 500 million gonococci per c.cm. are employed. The test may also be conducted by scarification, a technique used by Finkelstein and Gerschun (1913), who

employ two strengths of vaccine, the first containing 1 million and the second containing 10 million cocci per c.cm.; an area scarified with a vaccine prepared from other micro-organisms, e.g., streptococcus, serves as control. These observers corroborated the findings of Irons—that the reaction was more frequently positive in chronic than in acute cases—and in a few instances they obtained definite reactions even with the vaccine containing only 1 million cocci per c.cm. In 38 control cases definitely negative results were obtained in 35, while a feeble reaction was observed in 3.

The cutaneous reaction as a method of diagnosing gonorrhœa exhibits a considerable degree of specificity, but, like all skin reactions, its interpretation presents some difficulty. It is usually negative during the acute phases of the infection, is not necessarily positive during the chronic phases, may become negative when the infection is generalized, and is not absolutely specific.

In vitro *Serological Tests.*

Agglutination.

As one would expect, in view of the superficial and circumscribed character of the lesions, and of the small area involved in the disease, there is but little serological response in gonorrhœa. Nevertheless, when the disease has progressed for some time, and especially when lesions involving the pocketing of pus have developed, there is a fair degree of serological reaction, and in such circumstances the blood-serum of the patient may agglutinate suspensions of the gonococcus. Thus, in cases of epididymitis, Wildbolz (1903) and Baermann (1905), both demonstrated agglutinating antibodies. Such response, however, is uncertain and cannot be used either for diagnosis or for the control of treatment in gonorrhœa.

Complement fixation.

Question of a suitable antigen. Among the first applications of the test was that made by Müller and Oppenheim (1906) for the diagnosis of gonococcal arthritis. In the same year Brück (1906) applied similar methods to the investigation of the serum of persons suffering from salpingitis, epididymitis, urethritis, and recurrent iridocyclitis. The antigen used by this author was an extract of cultures of gonococci. Later investigators were for some time led by the apparent serological heterogeneity of the gonococcus to employ a mixture of several strains chosen at random for this purpose, but recently, mainly as a result of the work of Hermanies (1921), Gordon (1921, personal communication), Tulloch (1922), and of Torrey and Buckell (1922), it has come to be recognized that the majority of gonococci—roughly 70 per cent.—are so closely related to one another serologically, that one is justified in speaking of a ' predominant type ' gonococcus.

As certain strains of this predominant type are highly generalized in their serological relationship to other gonococci, the use of such strains

for the preparation of antigen greatly simplifies its technique and permits of the test being conducted under optimum conditions. In the preparation of the actual antigen employed, two objects must be kept in view :

(*a*) The material should exhibit a maximum degree of sensitivity, and
(*b*) It should not be unduly anti-complementary.

Various methods have been suggested to attain these ends. Thomson (1918) dissolves the suspension of cocci in alkali and subsequently neutralizes the product. This method has the apparent advantage that the antigen may consist of material in a fine state of division, so giving a large surface, which is probably of some importance in determining its sensitivity. On the other hand, the method is open to the criticism that a very slight variation in acidity or alkalinity seriously affects the specificity of all tests in which hæmolytic complex constitutes the indicator of complement fixation.

Extraction with fat solvents and subsequent digestion by ferments has been suggested, and this, according to Douglas and Fleming (1921), enhances the specificity of the complement-fixing qualities of antigen, at the same time reducing its non-specific anti-complementary qualities, at least in the case of *B. typhosus*. With the gonococcus, although the procedure does reduce the non-specific anti-complementary action of the antigen, it also, and equally, reduces its specific fixative qualities. A technique combining incomplete extraction by fat solvents with exposure of the suspension to a temperature of 80° C. has been suggested by M. A. Wilson and colleagues (1916–19), and by Smith and Wilson (1920).

While it is possible that preliminary treatment of the cocci by methods such as these may have certain advantages, the writer is in agreement with Kolmer and Brown (1914), that a simple suspension of gonococci in physiological salt solution constitutes the most satisfactory antigen.

If it be felt that the use of a single strain predominant type antigen is liable to give an undue number of false negative reactions, the best plan is to follow a suggestion of Torrey and Buckell (1922), viz. to conduct each test in duplicate, using for one test an antigen prepared from the predominant type, and for the other test one prepared from a number of ' irregular strains '. This is certainly a more rational procedure than is the employment of a compound antigen whose constituent strains are of unknown character and relationships.

The results obtained with the complement-fixation reaction. The following figures indicate the results obtained on using the complement-fixation test in the investigation of sera from cases attending the Venereal Diseases Clinic of the City of Dundee, but attention must be called to the fact that the technique employed was not the same as that advised in Vol. IX. A method elaborated by the writer and described in 1924, was used instead.

The total number of cases in which the results of clinical and microscopical investigation are available as a control of serological findings is to date 1,548. In this series the correlation of these methods shows :

Complement-fixation, positive ;	microscopical findings, positive ..	433
,, ,, , negative ;	,, ,, , negative..	737
,, ,, , positive ;	,, ,, , negative..	163
,, ,, , negative ;	,, ,, , positive ..	215

There are, therefore, 378 discrepancies between the findings of the two methods. This discrepancy, amounting to 24·4 per cent. of the whole series, is so formidable as to cast doubt on the practical value of the method, but closer enquiry concerning cases in which discrepancy occurred reveals the following :

(1) Considering the 215 discrepancies in which the microscopical findings were positive and the fixation negative—which would indicate that the test is not sufficiently delicate—it is to be noted that in 175 cases the disease was of duration not exceeding 14 days. In 19 instances the infection was of longer duration than 2 weeks, but of less duration than 2 months. In 8 instances a period of from 4 to 6 months had intervened between the taking of the specimen for microscopical examination and the performance of the fixation test. In 11 instances this period exceeded 8 months. One might say, then, that there were 27 instances in which a positive result was not obtained, although it might have been expected. An error for the whole series of 1·7 per cent. on the negative side.

(2) The discrepancies, 163 in number, in which microscopical findings were negative and the fixation test positive, are much more important, for they could be regarded as instances in which the test gave false positive results. A closer inspection of these cases, however, reveals the following : 7 were female cases in which the husband was known to be suffering from gonorrhœa ; 77 were female cases in which the microscopical examinations might be regarded as incomplete in that smears were not taken from all those parts of the genito-urinary tract that might be infected ; 26 were male cases in which prostatic massage revealed pus in the prostate, although no gonococci were found ; 46 were male cases in which the only microscopical examination made was that of the centrifugalized urinary deposit, and presumably, therefore, the majority of these cases were cases of chronic gleet ; 7 were male cases with purulent urethral discharge, in which, however, no gonococci were demonstrated. If we omit the male cases in which examination of urine was the only microscopical test performed, it is significant that of the remaining 117 no less than 84—71 per cent.—were females all of whom had been investigated either because a definite history of gonorrhœa was obtained or that disease was strongly suspected. The figures quoted indicate that the test as performed is fairly specific, and that it is as delicate as is compatible with accurate working. In 1,364 instances the Wassermann reaction was carried out at the same time as the complement-fixation test, and on comparing the results of

these it was clearly demonstrated that the one did not influence the other, a possibility that is of some importance in view of the frequent occurrence of syphilis and gonorrhœa in the same patient.

Methods of conducting the complement-fixation test in gonorrhœa and embodying the same principle as those dealt with above have been employed recently be Cohn and Gräfenberg (1925), by Cohn (1925), by Van der Hoeden (1926), and by Zoon (1928), who all obtained results closely approximating those quoted above.

Value of the gonococcus complement-fixation test. The observations dealt with in the previous subsection indicate that the test is of limited value only and it is improbable that it will ever occupy the same important position in the diagnosis and control of gonorrhœa as does the Wassermann reaction in the diagnosis and control of syphilis. Put briefly, a positive reaction is of significance, but a negative reaction is not. The interpretation of a positive result after vaccine treatment presents difficulty, as the reaction may persist for a prolonged period, and in such circumstances one cannot determine whether it is due to persistence of infection or to the antibodies called forth by vaccine treatment. The highest percentage of positive reactions—over 80 per cent.—is obtained in cases where generalization of infection has led to the occurrence of metastatic lesions such as gonorrhœal arthritis. When the infection remains limited to the genito-urinary tract the occurrence of positive reactions is to some extent determined by the degree of involvement of the various glands connected with it. In complicated cases of anterior and posterior urethritis in the male it is usual to obtain a negative reaction, but when the prostate or epididymis is involved a positive reaction is obtained in about 60 per cent. of cases, while in acute exacerbations of the infection with involvement of these structures, 80 per cent. of positive reactions may occur.

In females the findings are of a similar nature and positive reactions are obtained in similar circumstances. A low positive reaction rate when only the cervix is involved and a positive rate of 60 per cent. in cases of pyosalpinx, and involvement of the glands of Bartholini, negative reactions being usual in cases of urethritis.

Immunization.

The fact that gonorrhœa relapses so frequently as a local infection of the genito-urinary tract indicates that in certain circumstances, the nature of which is so far unknown, solid immunity does not necessarily result from an acute attack of the disease. Indeed, although the majority of cases of gonorrhœa clear up under—or, perhaps, in spite of—treatment, there is really no evidence that the process of cure depends on a general immunity response. The nature of the infection, then, is such as to lead one to suspect that the local reaction of the invaded area is of far more significance in the process of cure than is general reaction leading to the development of general tissue immunity.

None the less, experimental immunization of animals with a view to the production of anti-gonococcal sera for conducting serological tests, or for treating the disease, is frequently carried out, and there is abundance of evidence that the serum of such ' immunized ' animals contains antibodies—agglutinating, bactericidal, and opsonizing qualities—demonstrable *in vitro*, but, as no experimental animal is susceptible to infection with the gonococcus, evidence is lacking that such immunization really leads to the production of solid immunity. The value of large-scale applications of immunization in gonorrhœa can, therefore, be assessed only by clinical observation, a method which is always open to serious criticism owing to the lack of adequate control and the variability of the factors which influence the results obtained.

The Production of Anti-gonococcal Serum by immunizing Animals, and its Therapeutic Use.

Apparently, Rogers (1906) and Torrey (1906) were the first to produce anti-gonococcus serum upon a scale sufficiently large to permit of its application in the treatment of gonorrhœa in man, but in the same year Bruckner, Cristéanu and Ciuca (1906) treated experimentally gonococcal septicæmia of laboratory animals by injections of serum obtained by immunizing a horse.

The animals employed by Torrey and Rogers were goats, horses and rams, and definite therapeutic effects were said to be obtained by the use of the serum in the treatment of arthritis, prostatitis, and epididymitis, but the results in cases of urethritis and conjunctivitis were disappointing. These authors gave a dose of 2 c.cm. of their serum every second day, and in cases of arthritis marked benefit was said to result, but it would seem, if experience with other antibacterial sera be any guide, that the doses at first advised were inadequate, and in place of 2 to 3 c.cm. administered *sub cutem*, doses of at least 30 to 50 c.cm. would be required, preferably administered intravenously.

Among those who claim to have obtained satisfactory results from the employment of anti-gonococcus serum may be cited Uhle and Mackinney (1908), Herbst (1909), Belfield (1909) and Corbus (1914) ; but Butler (1908), Fletcher (1909), Brück (1909) and Waeber (1913) all report that the results were disappointing.

This sharp division of opinion is almost to be expected, as, so far, we have not at our disposal an adequate method of testing the qualities of the product before its use therapeutically. Until some method is devised whereby its value may be assessed experimentally, the question of dosage remains one of extreme difficulty and comparable results cannot be obtained by different observers. Debré and Paraf (1913) did suggest a method of standardizing anti-gonococcus serum which was based on the production of ophthalmitis in the rabbit. When doses of 200 to 300 million gonococci are injected into the anterior chamber of the eye of a rabbit, a marked inflammatory reaction takes place, and the dose of

anti-gonococcus serum required to prevent the occurrence of such reaction may be used as a guide to the probable potency of the serum for therapeutics. The same authors claim to have obtained satisfactory results by the intra-urethral administration of serum in two cases of acute urethritis, and report favourably on the treatment of gonococcal arthritis by its intra-articular administration ; they claim that of 15 cases so treated 14 were cured within a fortnight. It seems probable that the field in which anti-gonococcus serum promises to play its most valuable part is the treatment of complications of the disease, notably the more severe forms of arthritis and other metastatic lesions.

Autoserum Therapy.

Autoserum therapy has been suggested in gonococcal infections, and several authors have reported upon it, especially in arthritis. Wagon (1916) found it valuable, as also did Dufour and Debray (1920). In most instances autoserum therapy consists in removing the inflammatory exudate from the lesion in arthritis, orchitis, &c., and re-inoculating it *sub cutem*. When such a method is employed it is difficult to be assured that much of the benefit derived is not due to the effect of tapping the lesion.

Vaccine Therapy.

Vaccines have been employed far more than serum in the treatment of gonorrhœa, and it is in this connection that the question of immunity has received most attention. It should again be emphasized that the very fact that an individual may be infected with gonorrhœa several times in his walk through life shows that a general tissue immunity is of minor significance in determining the cure or preventing the occurrence of the disease. In the majority of cases the invasion is circumscribed, is limited to a surface, and although generalized intoxication occurs, is unaccompanied by the development of a general immunity response. In this respect infection with the gonococcus is unlike infection with *C. diphtheriæ*, which is limited in a similar way. One would expect, therefore, that beneficial effects from vaccine therapy of gonorrhœa are to be sought for, not in a rapid amelioration of the condition at the site of primary infection, but rather in the prevention of complications due to the extension of the infection by continuity or metastasis, and in the treatment of such complications.

Preparation of Gonococcus Vaccine.

The simplest vaccine consists of young cultures of the organism either grown in fluid medium or washed from the surface of solid media by saline, and killed by exposure to 50 to 55° C. for 30 minutes, or allowed to die off by undergoing autolysis either at room temperature or at 37° C. It is claimed by some that such simple procedures give vaccines which have maximum antigenic qualities. On the other hand, it has been

argued that they are unnecessarily toxic, and methods were, therefore, introduced for the purpose of reducing their toxicity ; one of the earliest of such methods was sensitization of the vaccine by the method of Besredka. M'Donagh and Klein (1912, 1913), employing such sensitized vaccines, claimed them to be of considerable therapeutic value and devoid of toxic qualities, provided that human serum was used for sensitizing the suspensions. Cruveilhier (1913) also employed them and commented favourably upon them, as did Dopter and Pauron (1913).

In 1913, Nicolle and Blaizot introduced a vaccine which was claimed to be even less toxic than ordinary vaccine sensitized by Besredka's method. The procedure was to cultivate the micro-organism on a special medium. Several authors reported favourably upon this preparation, which is known as ' Dmegon ' ; the initial dose was 25 million gonococci along with the commoner organisms of secondary infection prepared in the same way.

Digestion of the cocci has also been suggested as a means whereby their toxicity may be reduced, and Hirschfelder (1913) employed a suspension of gonococci digested with 2 per cent. pancreatin, which was subsequently neutralized and filtered, the filtrate being used as the vaccine.

Methods of detoxication which have received considerable attention of late years are those described in a series of publications by Thomson (1919, 1921). The first of these was to dissolve the suspensions with alkali and thereafter to neutralize it, but this was subsequently modified, as it seemed probable that the optimum vaccine was not obtained by the original procedure.

Thomson's later processes involved mechanical disintegration of the cocci, the material thus obtained being treated with various reagents so that it could be separated into acid-soluble, alkali-soluble, chloroform-soluble and water-soluble fractions. By suitable mixtures of these, a vaccine of minimum toxicity was obtained so that very large doses, representing from 5,000 million to 80,000 million cocci could be given without untoward effect.

Topical Application of Vaccines.

The topical application of vaccines has been suggested mainly as a result of the work of Besredka (1921), who observed that ' cuti immunization ' could be employed as a procedure for prophylaxis against anthrax. Since then the method has been applied to numerous other infections, digestive, respiratory, &c., and has also been extended to the field of therapeutics. In gonorrhœa, therapeutic application has recently been reported by Lambkin, Dimond and Robertson (1927), who, by cultivating the gonococcus on special media, obtained cultures possessing metachromatic granules. Vaccines prepared from such cultures are said to be especially suitable for topical application. It is too early yet to express any opinion as to the value of the method as at the time of writing adequate statistics have not been published.

Dosage of Vaccine in Treatment of Gonococcal Infections.

Acute urethritis. Although vaccines have been given in the treatment of acute urethritis there is really no evidence that they influence its course. The first dose of ordinary vaccine in such cases should not be more than 100 million, although in some instances even so small a dose as this causes marked focal reactions, and it may be necessary to employ very small doses—5 million—as an initial inoculum. The vaccine may be given at intervals of about five days, the dose being gradually increased, the rate of such increase being determined by the reactions of the patient—general, focal and local—until in some cases doses as high as 25,000 million are tolerated. The dosage, when detoxicated vaccine is used, is much higher, initial doses of from 3 to 5,000 million being usual.

Chronic urethritis, vaginitis, cervicitis, salpingitis, epididymitis, and arthritis. The dosage advised in such cases, when ordinary vaccines are used, has varied from 5 to 10 million in the earlier days of vaccine therapy, to the very much larger doses which are now employed; 200 million as an initial dose is now quite common. It is claimed by some, e.g. Sézary (1921) that general febrile and definite focal reactions should be aimed at in order to obtain the maximum therapeutic effect.

Route of administration of gonococcus vaccines. Gonococcus vaccines may be administered subcutaneously or intramuscularly when the ordinary type of vaccine is used, but when detoxicated vaccines are employed, intravenous inoculation has been suggested as a better method. The initial dose consists of 20 million detoxicated cocci, and, rapidly, at about 5 days' intervals, the dosage is increased to as much as 10,000 million or even 30,000 million.

The Value of Vaccine Therapy in Gonococcal Infections.

It is extremely difficult to assess the value of vaccine therapy in gonorrhœa as the method cannot be controlled by laboratory investigation, and we are, therefore, compelled to rely upon clinical observation. It may be said that there is little evidence that vaccines influence the acute phase of the illness (Pollock, 1909; Hartwell, 1910). As to their value in the treatment of chronic cases and of complications, Cole and Meakins (1907), Ohlmacher (1907), Aronstam (1908), Ballenger (1908), Irons (1908), Kinnear (1908), and others, all report favourably. It seems, however, that the main use of vaccines in the treatment of gonorrhœa is to establish a basal immunity early in the infection, not with a view to cure, but to prevent complications.

Non-Specific Protein Shock Therapy.

As in other conditions, attempts have been made to treat gonorrhœa by protein shock. A variety of reagents have been used for the purpose. Sterile milk (Hesse, 1918; Weiss, 1919; Gellis and Winter, 1918–19; Trossarello, 1920; Tansard, 1924; Hagen, 1925) has been the reagent most frequently employed, but solutions of casein (Morini, 1921) and typhoid vaccine (Cusson, 1919) have also been essayed. The influence

which protein shock therapy exerts upon gonococcal infection is even more difficult to determine than is that of vaccines. It certainly has no marked effect on the acute phase of the urethritis, and it would seem that any influence upon the complications of gonorrhœa is of the same nature as that which it appears to exert upon such conditions as rheumatoid arthritis.

Drug Treatment, Local and General.

Although topical application of antiseptics appears to be of some value in preventing gonorrhœa, the results of treatment by these, or by the use of drugs which are supposed to have a general action, are frankly disappointing. Notwithstanding the susceptibility of the gonococcus to all bactericides *in vitro*, the topical application of the same bactericides to infected mucous membranes is no more successful than is similar treatment of infections due to other and much less susceptible bacteria. Intravenous administration of silver salts has been attempted (Crohn, 1918 ; Sommerfeldt, 1918 ; Nast, 1919 ; Hamonic, 1919 ; Hofmann and Mergelsberg, 1921 ; Voormann, 1921 ; Sachweh, 1923 ; and Langer and Peiser, 1924). The danger of the procedure cannot be ignored, and was commented upon by Menzer in 1911 and again referred to by Romeick (1920). The intravenous administration of aniline dyes, notably acriflavine, has also been essayed (Wood, 1923 ; Jacob and Verasingam, 1924), but the value of these methods is somewhat doubtful.

Gonorrhœa as a Public Health Problem.

The most readily available statistics dealing with this are, for the most part, of German origin, and it is doubtful if they are applicable to other countries. Nevertheless, because of their accessibility, free use is here made of these statistics, largely taken from the article by Koch and Cohn in the ' Handbuch der pathogen Mikroorganismen ' of Kolle und Wassermann, Third Edition, 1927.

There are three features of gonorrhœa that call for special comment in this connection, namely, its wide distribution, its protracted nature coupled with the lack of any adequate test of cure, and the resultant extensive reservoir of infection constituted by the ' chronic carrier cases '.

In addition thereto must be noted the fact that gonorrhœa in women often remains undiagnosed, passes through acute phases, and goes on to the chronic stage, the patient remaining infective for prolonged periods without ever having been treated by a physician. Indeed, because of this, statistical enquiry concerning its incidence amongst women gives figures of but little value, so that it is claimed by some that the most useful index of the prevalence of gonococcal infection in the female population is the incidence of ophthalmia neonatorum.

Koch and Cohn (1927) state that the incidence of ophthalmia neonatorum is given by various authors as follows : Lomer, 28 per cent. ; Oppenheimer, 27 per cent. ; and Schwartz, 12·4 per cent. According to

Peller (1913) 30 to 40 per cent. of married women are victims of gonorrhœa as reckoned by the incidence of gonococcal conjunctivitis of the new-born. This finding appears to be borne out by the statistics of Oppenheim and Neugebauer (1914), which indicated that in certain groups of society the incidence among the male adult population was about 1 in 3. The importance to the community of such high percentages of infection is that before the introduction of the prophylactic measures advised by Credé approximately 60 per cent. of all cases of blindness were due to gonococcal ophthalmia. At a later date, Peller (1913) estimated that between 1 in 3 and 1 in 5 of the inmates of institutions for the blind, and 1 in 6 to 1 in 16 of the total blind population, had been deprived of their sight by that disease. It is to be observed, however, that many of his cases dated from the period prior to the introduction of Credé's prophylaxis.

The incidence of gonorrhœa in relation to social status, occupation, &c., is difficult to determine, there being numerous factors which tend to complicate statistical enquiry of this nature, while even those statistics which deal with whole populations can be regarded only as of relative value.

As to the age incidence of the disease, Erb (1906, 1907), enquiring into its occurrence among married men, found that of 386 cases, 85 per cent. acquired the malady before their twenty-fifth year, 11·5 per cent. in the age period between 25 and 30, and 4 per cent. at greater ages. Koch (1912) again dealing with a special group of society, namely, the German army, placed the incidence per annum at 1·5 per cent. to 1·7 per cent., and stated that it is as high as 3 per cent. in the Austrian and French armies. Peller (1913) concluded that from 10 per cent. to 12 per cent. of men between 20 and 30 years of age acquired gonorrhœa, and that about one-half of them suffered from the disease more than once. Fischer (1926) observed that of those attending the clinic at the Krankenhaus Südufer, Berlin, 26 per cent. showed evidence of gonococcal infection although attending the institution for other conditions, while Voights (cited from Fischer), during the immediate post-war period, observed 27 per cent. of cases of gonorrhœa amongst his married patients whose illnesses were such that they were not confined to bed. Gonorrhœa and especially its *sequelæ* and complications, is important as a factor responsible for the causation of various disabilities largely of a surgical nature and seen especially in gynæcological practice.

The influence of gonorrhœa upon the birth-rate is also important. Fischer (1926) cites Burkhart as estimating that 33 per cent. of childless marriages are due to gonorrhœa, while Prinzing, quoted by the same author, places the figures as high as 40 to 50 per cent. Erb stated that of 400 married women 4·25 per cent. were infected by their husbands ; but this figure is certainly too small, and later investigations indicate that it is certainly 15 per cent.

Finally, note must be taken of the results of introducing gonorrhœa into a population which has previously been free of that malady, and

information on this point is given by Bay-Schmith (1924) concerning an outbreak introduced into Greenland in 1914 by one man. It was observed that, without any measures being taken to deal with the malady, six years later reduction in virulence had occurred, so that the number of new cases was less, and, although the reduction was not regular, it was certainly notable.

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CHAPTER V. THE MENINGOCOCCUS.

By E. G. D. Murray (University of Cambridge).
With a Section by
W. Bulloch (London Hospital).

Introduction.

It is difficult to appreciate the point of view of an infecting organism, but much can be gained by realizing that its life is a hazardous one, and that even after successfully struggling to gain its first foothold, it is by no means certain of ultimate success. The ever-changing character of the environment in which it is constrained to live is largely due to the reaction which the parasite excites in the host, and as a general rule, the object and trend of the host's reactions are interpreted as purposively protective. As such they put difficulties in the way of the infecting organism, often only overcome by its great adaptability. But it must be recognized that certain of these changes may favour the parasite to a varying degree, according to the circumstances. Therefore, even though the subject is approached from a purely bacteriological point of view in this chapter, it is necessary to consider certain of the reactions on the part of the host, though only in so far as they affect the life and behaviour of the meningococcus.

History.

By W. Bulloch.

This organism was first described by Weichselbaum (1887) in Vienna, in six cases of primary cerebrospinal meningitis. It was a coccus usually in pairs and flattened on opposing surfaces. Individual cocci varied in size and in their affinity for stains. They were Gram-negative, and were very frequently found inside polynuclear cells. For these reasons Weichselbaum gave the name *Diplococcus intracellularis meningitidis*. Pure cultures presented certain peculiarities. There was no growth at room temperature but only at body heat. The vitality of the cultures was such that subculture was found to be necessary every few days. Weichselbaum carried out inoculations on 23 mice, 6 guinea-pigs, 6 rabbits and 3 dogs. Injection of pure cultures into the pleural cavity of mice invariably caused death, with the formation of a considerable pleuritic exudate, in which the cocci were numerous. The spleen was enlarged. Three rabbits injected under the dura mater died within 1 to 12

days, and 3 dogs inoculated in the same manner died with pachymeningitis, leptomeningitis, and encephalitis. Weichselbaum's findings were confirmed by Goldschmidt (1887) in one case, but it was not till 1895 that interest in the bacteriology of meningitis was renewed by Jaeger (1895). In an epidemic of twelve cases of cerebrospinal fever in the Stuttgart garrison he found a coccus agreeing in some points with the description given by Weichselbaum, but different in other respects. Jaeger's coccus was Gram-positive in cultures, but Gram-negative in the tissues. Occasionally it showed a capsule and exhibited a tendency to form chains. The vitality of the cultures was much greater than had been reported by Weichselbaum. A great many confirmations of Jaeger's findings were published, but these were very adversely criticized by Weichselbaum (1903). By degrees the correctness of Weichselbaum's assertions was confirmed by the researches of Councilman, Mallory and Wright (1898) in the Boston epidemic of 1897, of Lingelsheim (1906) in the great epidemic in Silesia 1904–5, of Bettencourt and França (1904) in Portugal, and of Elser (1905–6), and Elser and Huntoon (1909).

The existence of different serological types of meningococci was noted by Arkwright (1909), Dopter (1909) and Elser and Huntoon (1909), and later was very extensively studied by others.

W. B.

Nomenclature.

The differentiation of cerebrospinal fever from other forms of meningitis dates from the discovery by Weichselbaum (1887, 1903) of the causative organism, which he named *Diplococcus intracellularis meningitidis*. This name describes two characters of the micro-organism and the more important of them is the tendency to infect the meninges. It is perhaps regrettable that The Society of American Bacteriologists have chosen *Neisseria intracellularis* instead of *N. meningitidis* as the name they suggest for international adoption, since the intracellular position is not a site of predilection, but is related to the degree of virulence of the strain involved. The most commonly used name is meningococcus, and convenience and custom justify it being used throughout this chapter.

The names Parameningococcus (Dopter, 1909[1 & 2]) and Pseudomeningococcus (Kutscher, 1906) should no longer be used; they arose out of the earlier attempts at serological differentiation of the meningeal strains of meningococcus from otherwise indistinguishable cocci found in the nasopharynx, and many of the latter cocci are now included amongst recognized meningeal strains. The term 'Parameningococcus' is used in America, without any relation to Dopter's strains. It is used, together with the terms 'normal meningococcus' and 'irregular meningococcus', to indicate certain crude serological groups, and as such they will be discussed later. The use of these terms does not seem to be necessary to-day, and by discontinuing them much confusion will be avoided in the future.

Distribution.

The meningococcus is only found in the exudates, secretions and body fluids of man. It is most commonly confined to the nasopharynx, where it excites a catarrhal condition (Albrecht and Ghon, 1901 ; Westenhoeffer, 1905, 1906), with a muco-purulent discharge so copious and viscid in certain cases that it calls for special remark by the patient (Netter and Debré, 1911), but most commonly it does not cause any apparent inconvenience. Except in the cases accompanied by coryza it is very seldom isolated from the anterior nares, the pharynx, or saliva, and, in fact, certain organisms common in the latter situations appear to be definitely antagonistic to it. Thus, Colebrook (1915) noticed that a culture of a pneumococcus which replaced the meningococcus in the nasopharynx of a carrier inhibited meningococcus growth *in vitro*, and Gordon (1916) found normal human saliva inhibitory to meningococcus growth in culture, so long as it contained the normal salivary bacteria in a living state. Also Netter and Salanier (1917) believe that the replacement of the meningococcus by the pneumococcus in cases of meningitis, reported by themselves and others, is due to an antagonism of which the meningococcus is intolerant.

For the most part these nasopharyngeal infections are of short duration, and, according to Embleton and Peters (1915) and Clemenson (1918), persistent infections are due to some abnormality of the upper respiratory passages. Evidence has been collected by many authors which makes it certain that the meningococcus is present in the nasopharynx in every case of meningitis, at least in the early stages of the disease, and, according to Embleton and Stephen (1919), convalescent cases may remain very persistent carriers. Extension of the infection into the accessory air sinuses has been described a number of times, but extension into the middle ear is more uncommon. It is legitimate to regard infection of the nasopharynx as the normal form of the disease and to consider the more grave extension to the innermost cavities and fluids of the body as secondary and relatively uncommon accidents.

With generalization of the infection the subarachnoid space is the most noteworthy of the sites of localization. It is largely responsible for exciting the interest which has been taken in this organism, partly on account of the abrupt onset of the resulting disease and partly because of its occasional epidemic character. For this reason too the meningococcus is isolated most frequently from the cerebrospinal fluid.

Cases are cited in the literature of septicæmia without meningitis, or with meningitis as a late manifestation, and this form of the disease, which often lacks a definite clinical picture, may be more common than is shown by the records. Septicæmia would seem to be the rule in fulminant cases. Other localizations, such as in the skin, joints, pericardium, epididymis, &c., seem to be secondary to the blood infection. Various authors have described Gram-negative cocci in the papular rash,

petechial and purpuric patches, and Muir (1919) describes a case where the skin lesions seem to be due to emboli of meningococci in the smaller arterioles.

In the cerebrospinal fluid the cocci may be so abundant as to be seen in every field of a film, or they may only be found with some difficulty in films of centrifugalized deposits, or it may be necessary to incubate the fluid at 37° C. for several hours to find them. In some cases even cultural methods may fail with the first samples of fluid withdrawn, and the cocci only appear at a later stage of the disease. Like other Gram-negative cocci, there is a general tendency for the meningococcus to be intracellular, chiefly in polymorphonuclear neutrophil cells, and to this is due the typical description of them. However, very frequently the majority of cocci are extracellular, free in the fluid, and this condition is particularly associated with the most severe cases. At times spinal fluids are seen which are almost clear, having very few cells, but in which the cocci are very numerous indeed. According to Netter and Debré (1911) small poorly staining cocci become increasingly common in the exudate as the patient improves.

The intracellular position of the cocci is probably due to the readiness with which they are taken up by phagocytes. There is good evidence that the phagocytes are able to destroy the majority of cocci; this is particularly so of strains of low virulence (Murray, 1924, 1928); and where the cells hold what they have ingested those cocci no longer participate in the progress of the disease. But the cells are not always so successful and under certain conditions the cocci can survive phagocytosis. Freshly isolated spinal strains have a greater resistance to phagocytic destruction than have nasopharyngeal strains and stock cultures (Shearer and Crowe, 1917); cultures show more resistance to phagocytosis as their virulence increases (Murray, 1924). Thus it is, in meningococcal peritonitis in mice, that the more virulent the culture the greater is the number of extracellular cocci in the exudate, and the more readily they appear to regain their liberty after phagocytosis. Even so, the liberation of the cocci is complicated by other considerations: the extent to which individual cells are crammed with cocci, the total number of cocci present, and the numbers of cocci undergoing lysis and its degree (Murray, 1929).

Staining Reactions and Morphology.

The meningococcus is frankly Gram-negative, and, in my experience of strains I have kept for over ten years, there is no foundation for the statement made occasionally that old cultures tend to become Gram-positive. As a counter-stain 1 : 20 dilution of carbol fuchsine is most selective. The micro-organism stains well with the majority of the basic aniline dyes and with the products of oxidation of methylene blue, although it stains very poorly with ordinary preparations of methylene blue.

I have found that the sharpest differentiation of films of exudates are obtained with carbol thionine, a 1 : 20 dilution of carbol-gentian violet to which 0·1 per cent. glacial acetic acid is added. With these stains the cytoplasm of the cells are tinted while the nuclei and the cocci are deeply stained.

In form single cocci are round or ellipsoid, with little difference in the length of either axis, but when associated in pairs the opposed surfaces are flattened and the cocci are separated by a straight line. The association in pairs is so frequent that the term diplococcus has usually been applied to this micro-organism; tetrads are often seen, and are more common in certain cultures than in others. Chains are never formed. The cocci of a pair are always matched in size, although there is a great deal of variation between selected pairs or single cocci in the same specimen. This variation in size is more marked in cultures than in inflammatory exudates, and the extremes are in the region of 0·6 to 1·8μ.

In stained preparations individual cocci show a great deal of variation in the intensity of staining. In any preparation from a culture, a number of cocci have an appearance which has led to their being described as ' ghosts ', while a few stain much more deeply than the average and have been thought to be resistant, or more virulent forms, but without adequate evidence. It will be shown later that the cocci die easily in culture and it is due to autolysis of these dead cocci that the ' ghost ' forms appear. Autolysis is brought about by an endocellular enzyme which differs in essential characters from ordinary proteolytic ferments; particularly in that it will not attack *cooked* cocci; in being favoured by the action of lipoid solvents; and in its activity not being permanently interfered with at a temperature below 65° C. Calcium seems to be necessary to autolysis, which is inhibited by sodium citrate and potassium cyanide. The enzyme was first described by Flexner (1907), who showed that it acted upon organisms in no way related to the meningococcus.

Similar altered staining powers are seen in cocci in inflammatory exudates, but on careful examination slight differences in microscopical appearance can be made out between the ' ghosts ' seen in cultures and in pus. The changes seen in the intracellular cocci, in those which have just regained their liberty by rupture of a cell, and those which have no obvious relation to a cell, are all typical of intracellular bacteriolysis. Similar changes can be induced in cultures by extracts of polymorphonuclear leucocytes (Gengou, 1921; Murray, 1924) and they are very evident in the free cocci in exudates when phagolysis is well marked. It is improbable that autolysis takes much part in the degeneration of the cocci in inflammatory exudates, since dead cocci are so very readily ingested by leucocytes, and autolytic ' ghosts ' disappear within five to ten minutes when acted upon by extracts of leucocytes (Murray, 1924). It is also probable that the ' ghosts ' of cultures are dead cocci, whereas the ' granular degeneration ' forms in exudates are often viable.

However thickly packed a film may be, the cocci never appear to be actually touching, and in certain cultures the space intervening between unrelated cocci is well marked, but this appearance is only visible in thick films. Baker (1920) produced evidence which strongly suggests that the meningococcus possesses a definite capsule.

Cultural Conditions.

The meningococcus will grow quite readily *in vitro* so long as a few simple conditions are observed, and this is reflected in the literature, to some extent, by the great variety of media which have been recommended by different authors. Certain of these media are described in Vol. IX, to which reference should be made.

To obtain growth it is sufficient to use peptone-agar to which fresh blood (human or other) is added before it is allowed to set, or to smear the surface of the agar with a little blood. Instead of blood, fresh serum, ascites fluid or hydrocele fluid can be used in the same way.

Formal-serum agar, recommended by Nicolle, Debains and Jouan (1918), is easily made and gives good results. 0·2 per cent. of formalin is added to serum and followed shortly afterwards by 0·2 per cent. of 0·88 ammonia ; it is then diluted with twice its volume of distilled water and autoclaved. This formolized serum is then added to nutrient agar in the proportion of 1 in 3. Dilution of the serum before autoclaving may be omitted, with the advantage that less need be added, and dilution of the agar is avoided with no loss of efficiency. The serum does not coagulate when autoclaved, although it becomes ' smoky ' in appearance. When the autoclaved formol-serum has been added to agar subsequent autoclaving coagulates the serum.

Trypagar of Gordon, Hine and Flack (1916) gives good results, but it is difficult to avoid marked variation from batch to batch on account of the uncontrollable variation of Douglas trypsin broth which is the base of the medium. This medium requires the addition of fresh body fluids for primary cultures and freshly isolated strains.

E.L.D. agar has advantages over the above media : it is more constant : growth is rapid and viability prolonged : the yield is very large : and, above all, it maintains virulence. It is a modification of ' E.D.B. agar ' of Murray and Ayrton (1924) only in so far as half of the extract used is made from freshly killed sheep liver and the other half from freshly killed ox heart. It is important that the meat be collected immediately it is killed. The mince is extracted for 3 hours with distilled water (500 gm. to the litre) at 75 to 80° C., and the extract is filtered through a filter bed of the mince on muslin. The required weight of washed fibre agar is melted in a small part of the extract and then added to the remainder hot ; the whole is then heated rapidly at 95 to 100° C., after which it is allowed to set. In the extract the accessory growth factors are not liberated at temperatures below 75° C., and at higher temperatures than 80° C. they are adsorbed on the coagulum, but during the setting

of the agar they appear to be adsorbed on to the agar, and are retained by it when it is filtered or cleared. Until the agar has set the phosphates must not be allowed to precipitate, otherwise the accessory growth factors will be removed with them. To complete the medium the extract agar is remelted, the correct proportion of a separate preparation of tryptic digest of ox heart is added, together with 0·25 per cent. NaCl, 0·01 per cent. $CaCl_2$ and 0·02 per cent. KCl; the excess of phosphates are precipitated at pH 7·4 to 7·6, and the medium is filtered through paper pulp in a Buchner funnel. The reaction is then adjusted to pH 7·2 and the medium sterilized by autoclaving at 120° C. for 20 minutes. The only difficult procedure is the estimation of the correct concentration of tryptic digest, as this can only be done by determining the m.l.d. for mice of cultures grown on differing concentrations of digest. The amount required varies between 2 per cent. and 8 per cent. of good digests. If the digest is made in large quantities (6 to 10 litres) and the optimal virulence range titrated, there will be sufficient to last a considerable time, because it does not seem to alter with keeping (Murray and Ayrton, 1924). The readiness with which growth takes place is not interfered with by repeated autoclaving, and the initial care in making the extract is rewarded by the medium being as easily handled as ordinary peptone-agar. Primary cultures of the meningococcus and subcultures grow profusely on it, and so does the gonococcus if the reaction is made rather more alkaline, pH 7·8 to 8·0, without the addition of body fluids.

A critical examination of the details of the media used in relation to the biological characters of the growth obtained, shows that the meningococcus reacts to the conditions under which it is grown in a remarkable way. Quite small variations in any one selected constituent of a single type of medium can materially affect the culture in one or more ways: in its physical characters and appearance, in its rate of growth, in its viability, and even in its pathogenicity. No more than a brief review of the more important of these changes can be given here, but a more detailed examination of them is given by Murray (1929).

When touched with a wire the character of the colony is variously described: either that it picks up like paint and emulsifies readily in a drop of water, or that it is viscid, sticks to the medium and emulsifies with slight difficulty to give an even suspension. Murray and Ayrton (1924) have shown that these two descriptions, and yet a third, a granular type of growth with a dry surface and which gives a flocculent suspension, can be produced at will by varying the relative concentrations of sodium, potassium and calcium salts in the medium. Excess of either Na, Ca or K alone produced a sticky growth. Balancing Na against Ca gave smooth soft growth, excess of K in the presence of Ca gave the granular flocculent growth, while other combinations gave sticky growth. The optimal concentration of these salts finally chosen was 0·25 per cent. NaCl, 0·01 per cent. $CaCl_2$ and 0·02 per cent. KCl, and this gave a smooth creamy growth which picked up easily and suspended easily in water.

Potassium also influenced the viability of the culture, and its presence in suitable concentration allowed the culture to regrow over the surface of the medium from which growth had once been removed.

Growth is not obtained on ordinary media unless fresh body fluids are added, and, even then, it occurs more readily when sown direct from the cerebrospinal fluid than when subcultured, possibly because sufficient of the inflammatory exudate is carried on to the medium in the first instance.

For these reasons and on the evidence of D. Jordan Lloyd (1916, 1917) it is often believed that ' amino-acids are the essential food substances ' of the meningococcus ' and that the function of the vitamins in accelerating growth is exerted through their effect upon the protein equilibrium of the cell '. These conclusions are only partly true, and are misleading. McLeod and Wyon (1921), Wyon and McLeod (1923), Murray and Ayrton (1924) have shown that excess of tryptic digest of meat and certain amino-acids inhibit the growth of meningococcus, and J. Gordon and McLeod (1926) have shown that only 3 out of 14 pure amino-acids can be classed as favouring growth (taurine, aspartic acid and alanine) ; certain amino-acids are definitely inhibitory even in the concentration in which they may occur in the medium recommended by Lloyd. It is probable that nitrogen compounds of greater complexity than amino-acids are necessary for the nutrition of the meningococcus.

Another point of importance was demonstrated by Murray and Ayrton (1924) in relation to the virulence of cultures to mice. They showed that the killing power of cultures bore a direct relation to the concentration of the selected batch of tryptic digest of muscle added to the medium. By alternating the growth on two selected media the strain used would be alternately virulent and almost harmless (Murray, 1929) (see p. 304).

The addition of fresh body fluids to the medium has an undoubted stimulating effect upon the growth and its rate of onset. By varying the method of adding the fresh fluid and the subsequent treatment of the medium, results are obtained which justify the view that accessory growth factors play an important part. But this is not the sole action of the added body fluids, for it has been shown (Gordon and McLeod, 1926) that the addition of serum to a medium protects bacteria against concentrations of amino-acids which otherwise inhibit growth.

Fresh ascitic fluid or fresh hydrocele fluid favours meningococcus growth more than do serum or blood, when added immediately before use to medium cooled to 50° C. But when ascitic fluid or serum have been kept exposed to air for a short while, they become strongly alkaline, and lose their growth-stimulating power ; they may even become inhibitory. This leads to another factor in growth conditions which is of considerable interest, and depends upon the change in reaction due to metabolic processes. Phelon, Duthie, and McLeod (1927) have shown that the meningococcus grown in a slow current of oxygen rapidly develops an alkalinity in the medium which proves fatal to the culture, usually within 36 hours after the commencement of oxygenation. The neutralization of

this alkalinity, which can be effected by aerating with CO_2, prolongs the life of the oxygenated culture. The production of peroxides is discounted in this case by the active production of catalase by the meningococcus. Despite this the meningococcus is strictly aerobic in culture, but is not exacting about the concentration of its oxygen supply, and survives a reduced oxygen tension for a considerable time. The long life of cultures on Dorset's egg medium sealed with waxed plugs, may be partly concerned with the limited oxygen supply under these conditions, and oftentimes a culture which appears to be dead is revived if the waxed plug is replaced by an ordinary wool plug and subculture delayed till after a few hours' incubation.

Acid media, too, are unfavourable to meningococcus growth and the optimum reaction of media lies between pH 7·0 and 7·4. The optimal temperature for growth is generally accepted to be in the region of 36 to 38° C., and growth is arrested above 41° C. and below 27° C. At one time failure to grow at 23° C. was considered an important differential character of the meningococcus, but it proved unreliable because other allied organisms shared this peculiarity. The lowest temperature at which meningococcus growth takes place is influenced by the medium, the presence of fresh blood or gelatin being favourable to growth at low temperatures.

Drying of the surface of the medium is detrimental, and growth is obtained most readily on freshly poured plates or freshly prepared slopes cooled quickly. At the same time a too wet surface diminishes the yield. The exact requirements are difficult to determine and maintain, but Gates (1919) believes that the correct degree of moisture can only be maintained by the use of closed containers for the cultures.

Cultural Characters.

The Colony.

On an ordinarily suitable transparent medium the meningococcus colony has a characteristic appearance, and when 24 to 48 hours old it is remarkably regular in formation : a thin, transparent, perfectly circular colony of 1 to 2 mm. diameter, with a smooth moist surface of even though definite curvature, and an extremely regular and sharply defined margin. Viewed with a hand lens by transmitted light it is almost colourless, and the bars of the window can be focussed in the colony. Turning from a window to a neighbouring wall and looking through the colony it takes on, at a particular angle, a uniform pearl-grey lustre. By this light a blue, stippled or iridescent colony can be neglected as not being meningococcus. Under a low power of a microscope (2-inch objective) it has a central finely granular zone, and a peripheral perfectly clear refractile ring which may occupy as much as a third of the radius ; the regularity of the margin is emphasized by this magnification. With ageing the central zone tends to extend, to become more granular, and to

take on a slight buff tinge, but there is never any pronounced pigment. When touched with a wire it picks up easily, and when rubbed up in saline or water it gives an even suspension quite easily. Atkin (1923) describes characteristic appearances of aged colonies which he correlates with the agglutinable types.

Heavy inoculation on agar media yields a confluent smooth growth, which when scraped together into a heap varies slightly in appearance with the medium and the strain.

In fluid media growth is slow, and a thin even turbidity is produced with a deposit at the bottom of the tube with time. A fragile pellicle on the surface is not uncommon in old cultures.

On most media the life of the culture is short and frequent subculture is necessary to maintain the strain. The first few generations after isolation are the most delicate and thereafter the culture can be induced to grow on ordinary peptone-agar, though certain strains are more easily acclimatized than others. However, cultures die out very suddenly and unexpectedly whatever the medium and often are difficult to keep alive. In all cases cultures survive best when kept at 37° C. Dorset's egg medium gives a rapid, thin growth and seems to favour long survival of the culture. The relation of survival to medium and cultural conditions is discussed under ' cultural conditions ' and under ' viability '.

Fermentation Reactions.

There is general agreement that the meningococcus produces acid without gas in glucose and maltose, and that it does not attack lactose, saccharose, mannitol, mannose, dulcitol, inulin, raffinose, erythritol, inositol and glycerol. Its action on lævulose, galactose and dextrin was a matter of dispute, but the generally accepted view, based on the work of Elser and Huntoon (1909), is that these sugars are not attacked.

Freshly isolated strains have been observed to be capricious in fermenting glucose and maltose (Arkwright, 1909 ; Griffith, 1916 ; Scott, 1916) ; certain of them attack one more strongly than the other, while less commonly both sugars are attacked equally well. Occasionally strains are isolated from the cerebrospinal fluid which attack only one of these sugars, and, rarely, a strain which does not touch either. Usually these abnormal strains behave more typically after they have been kept in culture for a time. Irregular fermentation is more marked in strains freshly isolated from the cerebrospinal fluid than in the nasopharyngeal strains.

It has been observed that the acidity produced is evanescent when the attack on the sugar is slight, and this is possibly due to the power possessed by the meningococcus of producing alkali with liberal oxygenation, and in part, too, on account of the excess of serum or other body fluids added to the medium. Agar media give a more rapid reaction than fluid media, and the results are more easily read, but the production of alkali is also intensified. Fluid media suffer

from the disadvantage that growth is less certain, and, when obtained, a definite reaction is commonly delayed.

Whatever the medium used, the greatest possible care must be taken in preparing it, and it is worthy of notice that Fernbach, Schoen and Mori (1928) have shown that in very dilute alkali and at low temperature (40° C.) the optical rotation of hexoses (such as glucose and mannose) undergoes a change which makes these solutions optically inactive, and materially affects their fermentation by micro-organisms.

Viability.

Survival in Culture.

Although a ready and abundant growth of meningococcus is obtained on suitable medium, the life of the culture is short, and frequent subculture is necessary to maintian freshly isolated strains. The period of life of a culture can be materially influenced by the medium, as is shown by survival on Dorset's egg medium and on medium containing the correct balance of potassium salts. Nevertheless, the strain introduces a variation quite independent of these factors, and sudden death of a culture which has grown well is not an uncommon happening, and may be due to several factors already considered. The liability of cultures to die can be examined by microscopical and cultural methods on single colonies which are allowed to grow for several days at 37° C. As the culture ages, autolytic changes in the cocci of the central part of the colony become more abundant, and subcultures from that region become increasingly difficult, while the edge of the colony gives more typically staining cocci, and more ready growth on subculture. Within certain limits, the longer a strain has been in culture, the longer it will survive without subculture, but, on most media it is not safe to leave well-established strains for longer than ten days, with the exception of Dorset's egg medium in tubes with waxed plugs, on which most cultures will live six months and many over a year, and Murray's ' F ' medium under liquid paraffin, which is not so favourable for survival, but maintains virulence better.

Cultures must be maintained at 36 to 38° C., although under certain conditions the meningococcus withstands cold easily, but a not too heavy growth survives better than a profuse one, and this may be one of the reasons for success with the two media mentioned above.

When survival at different temperatures is investigated, one of the important factors is the density of the suspension ; with extreme cold the thicker the suspension the longer the survival, but in the region of optimal temperature for growth the reverse holds true.

At temperatures above 41° C. the meningococcus is easily killed, but the published time-temperature relations disagree somewhat owing to the variations in technique and the strain. Roughly the lethal time-temperature relationship for a suspension heated in a water-bath is: 45° about 3 hours, 50° about 1 hour, 60° about 15 minutes, 70° about 5

minutes, and 80° or higher a period which can be measured in seconds. Bulk, density of suspension and menstruum (broth, saline, &c.) are of the most important factors causing disagreement in the observations.

At low temperatures cultures survive surprisingly ; at 6 to 8° C. they will live for 1 to 3 weeks, and Flexner (1907) found continuous exposure to 2° C. of suspensions in saline more harmful than alternate cooling to 2° and warming to 37°. Extremes of cold between —10° C. and —78° C. for short periods such as twenty minutes appear to have little effect on the meningococcus, even if rapidly warmed after such an exposure (v. Lingelsheim, 1906 ; Murray, 1929).

Exposure of cultures to room temperature has given rise to considerable disagreement amongst authors. Here again the conditions of investigation vary according to place and time of year, medium, light, oxygenation, lengths of incubation before exposure, the strain and the length of time it has been isolated, &c., all of which are important. Without uniformity of the above conditions the longest survival times recorded vary between 5 days and 9 weeks. Von Lingelsheim's (1906) suggestion, that cultures live a short time at low temperatures only because they do not grow and the individual life of the coccus is short, probably involves the most important factor when the other lethal influences have been eliminated.

Light has a lethal action on cultures and diffuse daylight is less harmful than direct sunlight. In both instances there is a marked difference between strains. The survival time when exposed to direct sunlight bears a direct relation to both the time of year and the country, and it is possible that the temperature developed plays an important part in these results, although it is not recorded. The action of light may be influenced by the difference in filter properties of different kinds of glass used ; add to this the combined action of light and oxygen and the effect of light on certain of the enzymes concerned with oxidation and reduction processes of the cell, and the discrepancies in recorded observations might easily be accounted for, apart from any influence of the temperature developed.

The effect of drying the meningococcus has varied with the method used. To allow whole cultures to dry up invariably proves fatal, but this applies to many hardy bacteria too, and involves concentration of substances present in the medium at the time. When the growth is removed from the surface of the agar and dried, the meningococcus proves more resistant than it is generally supposed to be. Elser and Huntoon (1909) found that the age of the culture at the time of drying was important : two-days' old cultures were more resistant than either one-day or seven-day cultures, and seven-day cultures were more resistant than one-day. Growth removed from the medium and dried in a desiccator will survive for months and even retain virulence, whether dried whilst frozen at reduced pressure (4 mm. Hg) over P_2O_5 as recommended by Swift (1921), or simply over NaOH at reduced pressure (20 mm. Hg) at 37° C. as used by Murray (1924). The medium used for recovery of the dried organisms is very important and success depends upon it ; either adding ascites

broth to the dried bacteria (Elser and Huntoon, 1909) or smearing the dry powder over agar smeared with human blood (Swift, 1921 ; Murray, 1924) are satisfactory methods.

Flexner (1907[1]) showed that sodium chloride was directly injurious to the meningococcus, and that its effect was neutralized by calcium and potassium salts. Knowledge of the action of sodium chloride was carried a stage further by Shearer (1917), who observed that the meningococcus survived longer in distilled water, or in a 1·5 per cent. solution of pure NaCl, than it did in 0·85 per cent. NaCl at 37° C., and that the presence of KCl accelerated the protective action of $CaCl_2$ against the poisonous action of NaCl. The process involved seems to depend upon the dispersion of the proteins and other substances adsorbed or condensed at the surface of the cell to form its membrane.

Bile-salts have an effect on the meningococcus similar to that they have on the pneumococcus, though less intense (Dopter, 1921 ; Murray, 1929). As the meningococcus is an organism which very readily autolyses, it might be possible to make observations similar to those of Atkin (1926) on the relation of autolysis to bile solubility of the pneumococcus.

The meningococcus is rapidly killed by a number of substances in dilute solution and amongst the more powerful are formaldehyde, phenol, mercuric chloride, silver nitrate and menthol. Hydrogen peroxide is less rapidly effective. The vapours of certain essential oils are also poisonous to it, and are able to inhibit the growth of subcultures ; such are thyme, bergamot and mint, but the most effective is eucalyptus. M. H. Gordon (1920) and others investigated the disinfectant power of air heavily laden with droplets of zinc sulphate or chloramine-T solutions, and they observed that the disinfectant power was apparently confined to the actual droplets coming into contact with the cocci. Their attempts to free carriers by inhalation of such charged air were not attended with marked success, but they observed that the men most desirous to become free cleared up more rapidly than the others.

Survival in Inflammatory Exudates.

It has been found necessary to employ elaborate precautions, designed to protect the meningococcus from exposure to cold, drying and light, in order to ensure successful culture from the cerebrospinal fluid and the nasopharyngeal secretions from cases of meningitis and from carriers. Those who have had much experience have no doubt of the efficacy of these precautions. Thus it is recommended to plate the material immediately it is collected, and, if the patient is far from the laboratory, it is best to take the plates or slopes in a warm jacketed container rather than risk failure by bringing the swabs or fluid back to the laboratory. A method which has proved useful is to inoculate a small area of the plate fairly heavily with the material as soon as it is collected, and then, on returning to the laboratory, to incubate the plates for a few hours before spreading the material over the rest of the surface.

Cerebrospinal fluids sent by post give a markedly lower percentage of successful cultures than those examined as soon as drawn (von Lingelsheim, 1906, and others), and Netter and Debré (1911) found that fluids rich in organisms gave only sparse colonies after standing 6 to 10 hours at room temperature, and were invariably sterile after 18 hours. From what has been said of the isolated action of light, cold, desiccation, &c., on growth removed from the medium, the detrimental effects of exposure cannot be ascribed to these factors alone. They may nevertheless produce important changes in the inflammatory exudates themselves and in the body cells, and even differentially favour organisms other than the meningococcus which may be present, and these changes may in turn react upon the meningococcus. Thus, since the products of lysis of leucocytes are lytic to a marked degree for the meningococcus, the fluid may become sterilized by the leukines liberated through the breaking down of leucocytes under the conditions in which the fluid is kept. The more virulent strains show a greater resistance to phagocytosis than do the less virulent, but they also cause more damage to the leucocytes associated with them and so create unfavourable conditions for themselves. It must be noted, too, that the meningococcus is very susceptible to non-specific lysis by alexin, though cerebrospinal fluid, even from inflamed meninges, is poorly supplied with alexin.

Quite often great advantage is obtained by incubating the cerebrospinal fluid at 37° C. for some hours before making cultures from it. That is in so far as growing the micro-organism is concerned, but it has been shown (Murray, 1929) that cultures obtained from the fluid after some hours delay are less virulent than cultures obtained from the same fluid immediately it is drawn, whether or not the cocci present are increased or decreased in numbers. Attention is called to this because it may be a matter of considerable importance for the purpose of producing therapeutic sera to use only the most virulent strains.

Virulence.

The determination of the virulence of cultures has been admitted by most authors to be extremely difficult, and the results have been so inconstant from one experiment to another, although the same strain has been used, that it has been held to be impracticable (Dopter, 1921). One of the most important factors upon which success depends, both for the titration of virulence and for its maintenance in cultures, is the constitution of the medium (Murray and Ayrton, 1924). In experiments in which all other known factors have been kept as nearly as possible comparable, controlled variation of the concentration of tryptic digest in the medium has resulted in the cultures being highly virulent on one and harmless on another medium. The importance of this observation is emphasized by the change in virulence being reversible, through the simple expedient of subculture from one selected medium to another, and by the

constancy of the results given on any one medium (Murray and Ayrton, 1924; Murray, 1929). For example: a culture of meningococcus was plated on two batches of media with this result,

"EDB" Medium 86 Avirulent
"EDB" Medium 88 Virulent

Medium 86 Avirulent
Medium 88 Virulent

and this crossed effect was repeated several times in succession.

The choice of animal and the route of inoculation are important too, and in my experience intraperitoneal inoculation of mice gives satisfactory results. Other factors cannot be ignored (Murray, 1924, 1929) but their several claims to importance are matters of the degree to which it is desired to control the interacting systems.

The degree of virulence exhibited by different strains varies considerably, and although, from the point of view of virulence, the medium upon which a culture is maintained is of paramount importance, the medium on which the primary culture is obtained is less so. Certain types of medium accelerate attenuation by subculture (inspissated serum and certain agar media) whereas others tend to retard the process (ELD Agar of Murray and Ayrton, 1924, and egg), and it is fortunate that the latter also have the tendency to prolong the life of the culture. In the investigation of virulence it is important to obtain primary cultures, but it is also important that the cultures are sown as soon as possible after the withdrawal of the material from the body; with keeping even cerebrospinal fluid yields less virulent strains. There is very little evidence relative to difference in virulence between spinal strains and nasopharyngeal strains, but Heist and S. and M. Solis-Cohen (1922) interpret the results of their attempts to grow cultures in whole fresh human blood as indicating that the spinal strains are more virulent than the carrier nasopharyngeal strains.

The experimental exaltation of virulence of the meningococcus is always an uncertain and troublesome process. In fact, very few authors have claimed to have succeeded. It happens on rare occasions when a strain is passaged through animals, but more commonly the recovered strain is attenuated. Murray (1924) described an *in vitro* method whereby the meningococcus can be raised in virulence by subjecting the growth removed from agar medium to the destructive powers of extracts of leucocytes. The concentration of leucocyte extract relative to constant mass of culture is varied over a series of tubes, and from these tubes kept at 37° C. subcultures are made at short intervals of time (6, 12, 24 and 48 hours or longer). When the virulence of all these cultures is examined, it is found that there is a range of concentration of extract which yields cultures progressively increasing in virulence as the time of contact is prolonged, and that lower concentrations or higher concentrations (relative to mass of culture) yield cultures either unchanged, or lowered in virulence according to the circumstances. The effective time of contact

appears to be related to the lytic potency of the leucocyte extract, but even in favourable concentrations of powerful extract the virulence of recovered cultures will again decrease after a sufficiently long time of exposure. The method requires a little care, but it has the advantage of speed and certainty, although the degree to which virulence will be raised cannot be foretold.

It has been mentioned that the meningococcus survives desiccation under suitable conditions and it is interesting that this process does not appear to attenuate the virulence of the culture used.

The exact relation of virulence to endotoxin is still uncertain, but the available evidence tends to show them to be unrelated (Gordon, 1920, p. 45 ; Murray, 1929). Strains of low virulence with a high yield of endotoxin are found, but more commonly a low yield of endotoxin is associated with high or medium virulence. Exaltation or lowering of virulence does not appear to alter the quantity of toxin yielded by a strain but only affects the power of infecting animals.

Toxin.

Up to the present every attempt to discover a soluble toxin produced by the meningococcus has failed. It is significant that all the processes of cellular activity of this organism which have been investigated (hæmolytic substance, maltase, autolysin, enzymes concerned with oxidation and reduction, and toxin) with the possible exception of the proteolytic enzyme, appear to be endocellular and dependent upon the disintegration of the cell for their liberation.

There is hardly room for doubt that the meningococcus is possessed of a powerful endotoxin, although its complete investigation presents many difficulties yet to be solved. Its presence is clearly indicated by the fact that a very much larger dose of intact killed cocci is required to kill a normal untreated animal than of lysed killed cocci.

A number of methods have been used to liberate the endotoxin, and all of them have disadvantages for quantitative work, which are reflected in the inconsistent experimental results of their application. The more important of the methods used are autolysis, solution in 0·025 N NaOH, Besredka's well-known method, the osmotic pressure method, the bile-salt method, lysis by fresh guinea-pig serum, and lysis by leucocyte extracts (Besredka, 1906 ; Gordon, 1920 ; Murray, 1929). The comparison of these methods is exceedingly difficult, as no standard of measurement of endotoxin content of a culture is yet possible. Each method is liable to its own peculiar sources of error, and conclusions cannot be drawn from a single experiment, even though aliquot parts of the same culture are used in different ways. The main sources of error are the measurement of the dose, incomplete and inconstant disintegration of the cocci, incomplete dissociation of the endotoxin from the remains of the cocci, the addition of extraneous toxic substances, alteration of the endotoxin by the process

used and in the time taken to liberate it, and several other less active though far from negligible difficulties (Murray, 1929).

The properties of meningococcus endotoxin are hardly known, but it is remarkably resistant in some ways and equally fragile in others. It appears to be unaltered by desiccation. It shows considerable resistance to heating at 100° C. and over for 30 minutes, but it is destroyed within 2 hours. The loss of toxicity by heating is more evident when cultures of low toxicity are used than with those of high toxicity (M. H. Gordon, 1920). To some extent heating appears to aid the dissociation of the endotoxin from the debris of the cocci. Nevertheless, a degenerative process, expressed by loss of toxicity, occurs rapidly when the preparation is kept at room temperature in the dark. This degeneration combined with slow dissociation is possibly the cause of the irregularity in potency of different preparations made from the same strain under as nearly as possible identical conditions. It is interesting that the degenerated endotoxin can be reactivated by filtrates of meningococcus cultures, and that the reactivating capacity of the filtrate degenerates in a similar way to the endotoxin. Not all cultures and not all strains yield a suitably active filtrate, and the conditions of its activity have still to be discovered. However, the most active filtrates appear to be completely without toxicity (Murray, 1929).

Immunity.

Immunity Reactions in Infections of Man.

Meningococcus infection in man has two stages: (1) Infection of the nasopharynx, which in the majority of cases not only fails to progress but dies out within three weeks. Persons so infected are commonly spoken of as ' carriers '. (2) Generalization of the infection with secondary localizations in the tissues, organs and fluids of the body, particularly the subarachnoid space, skin and blood. Persons so infected are commonly spoken of as ' cases '.

There is very little evidence of the production of specific immune bodies by ' carriers '. Worster-Drought and Kennedy (1919) tested the serum of 25 carriers for agglutination against the prevalent spinal strains of the epidemic of 1915 in England, and against the carrier's own strain, with negative results. Heist and S. and M. Solis-Cohen (1912) showed the whole blood of carriers to be more inhibitory to growth of the meningococcus than is the blood of normal persons. This may be due to the production of immune bodies which escape notice by other methods of testing.

In ' cases ', on the other hand, specific antibodies have been demonstrated in the serum, although they do not appear in the cerebrospinal fluid.

In many instances where the agglutinating response of cases has been investigated, the information is only of limited value, since the prevalent

epidemic strains were neglected, and the negative results are therefore valueless. However, Worster-Drought and Kennedy (1919) not only used the prevalent epidemic type strains in their tests, but in many cases used the patient's own culture in addition. They show that the development of agglutinating properties is very irregular and may fail altogether. It bears no relationship to prognosis and commonly disappears during the course of the illness, and so fails to be of value for diagnosis in late stages of the disease, except when positive.

Opsonic reactions and bactericidal reactions are difficult of application, but there is evidence (Davis, 1905, 1907; Houston and Rankin, 1907) that the serum of cases is more active than normal serum. The serum of cases has been shown to fix complement in the presence of meningococcus antigen more strongly than normal human serum, but, most often (Bell, 1920) this reaction gives no indication of the agglutinating type of the infecting strain. Anti-endotoxin has not been shown to be produced in the course of the disease, but there is a suggestion that it is produced in the experiments of Davis (1907) in which killed meningococci suspended in saline proved very toxic to a normal man, whereas the same dose was not toxic to two meningitis patients although their blood was strongly bacteriolytic.

Serological Differentiation of the Meningococcus.

Agglutination Reaction.

In applying this test, especially when using the ' absorption reaction ', considerable attention must be paid to the technique employed. It would seem that the disagreements in the literature, particularly when related to a particular epidemic, can be traced to fundamental dissimilarities in the technique of different authors. A discussion of the general principles of the reaction employed will be found in Volume VI, but reference to a few points of special importance relating to the meningococcus may be made here.

In the first place the selection of suitable strains with which to immunize rabbits, or other animals, must be made with due regard to the reaction of the strains with the agglutinating sera produced by other strains. As there is some degree of cross agglutination between individual strains belonging to what are considered to be distinct agglutinable types, it is desirable, when differences are to be measured, to select as antigens only those which exhibit the greatest type specificity; but when relationship is being searched for, the reverse process of selection should be applied. The rabbits yielding the serum should be young and their course of immunization of short duration. Also it is advisable to use only sera which react consistently with several representative strains of their respective serological type.

In preparing the agglutinable suspensions young cultures should be used and condensation water should be excluded. During the first three or four weeks after it is made the agglutinability of the suspension is very

variable but after that time it acquires a stability which lasts about six months. Heating at 65° C. accelerates stabilization and destroys the autolytic ferment and so preserves the cocci. Their agglutinability is increased by heating between 50 and 70° C., when compared with the fresh state, but it is impaired at 80° C. Flexner (1907) observed that 1 : 20,000 KCN killed the meningococcus and preserved it from autolysis, and this might prove useful in preparing agglutinable suspensions without heating them ; it is a method which does not appear to have been tried. Phenol as a preservative after heating is useful, but formalin eventually causes spontaneous agglutination.

The reaction is not usually complete in less than 24 hours, and it takes place more quickly and more completely at 55 than at 37° C.

There is often with meningococcus sera a distinct antibody excess zone (' pro-agglutinoid zone ') which is most marked after a prolonged course of immunization.

As the outcome of tests done between the years 1906 to 1914, the agglutination reaction was considered unreliable, as the importance of agglutinable types had not been recognized. This literature is reviewed by Eastwood (1916) who clearly states the position at that time.

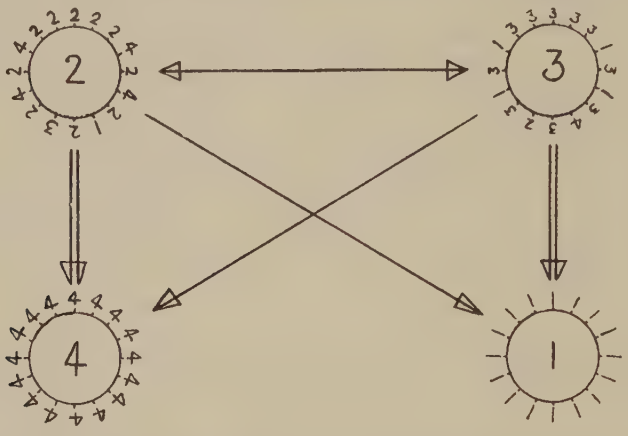
Dopter (1909) described as ' parameningococci ' nasopharyngeal strains which were only distinguishable from spinal meningococci by agglutination tests and he thought they might be capable of causing meningitis. At a later date, he and other workers showed that these ' parameningococci ' definitely did cause meningitis, and as the result of applying the absorption reaction he divided the organisms from cases of meningitis which he examined into meningococci and parameningococci α, β and γ (Dopter and Pauron, 1914).

Gordon and Murray (1915) applied the ' absorption of agglutinin ' reaction to spinal strains isolated during the then prevalent epidemic, and distinguished four agglutinable types. By the simple agglutination reaction these four types tended to fall into two main groups, one comprised Types I and III and the other Types II and IV. These findings were subsequently confirmed by a number of workers using Gordon and Murray's strains and sera, either directly or as co-ordinating links. Of these the series investigated by Fildes and Baker (1918) is particularly valuable, as it represents the whole of the strains isolated by these authors.

Pullon, working at Cape Town, and unaware of the work of Gordon and Murray, used absorption methods and identified three agglutinable types, A, B and C (personal communication, 1917). His strains were subsequently examined by Gordon, who found them to agree absolutely with types III, II and I of Gordon and Murray. A considerable weight of evidence confirmed the existence of these types in the 1915–19 epidemic. Varied evidence was brought forward : such as the similar serological relationship between the nasopharyngeal strains and spinal strains in individual cases, between carriers and cases in a locality, and between

carriers associated closely with cases ('contact carriers'); also the percentage of spinal strains identifiable as one or other type (98 per cent.) pointed to the importance of these types in that epidemic.

A definite relationship between the types was recognized by all workers by the cross agglutination between them and it is clearly represented in



the diagram here reproduced, which Fildes and Baker used to illustrate their findings. Thus it was shown that Types I and IV are unrelated; that a close relationship exists between Types I and III and between Types II and IV; that some degree of relationship can be found between Types I and II and between III and IV. This cross agglutination is not exhibited by every strain, and not with every serum, and it could be intensified or largely eliminated by a careful selection of the strains of each type used for immunizing. Moreover, as Fildes and Baker remark, it does not invalidate a classification based upon the purer members of the types.

Scott (1916, 1918) and F. Griffith (1916, 1918) found that the majority of strains could be arranged in two groups, although the degree to which individual strains agglutinated with any one serum varied considerably. They also found a correspondence between these serological groups and the grouping of the same strains according to the preference they showed in attacking glucose or maltose, an observation which Fildes and Baker were unable to confirm. By absorption methods Scott and Griffith subdivided their two main groups into a number of subgroups, and a careful scrutiny of their tables gives the impression that their results are not so widely divergent from the findings of other authors as has been supposed.

A. S. Griffith (1920) re-examined a number of strains classified by Gordon, and agreed that the typing held good if care was exercised in selecting the immunizing strains. He also examined selected strains reported on by F. Griffith, chosen because of the difficulty they presented of classification, and found 51·6 per cent. were completely identified with one or other of Gordon and Murray's four types, 9·7 per cent. showed

cross relationship to two types, and 38·7 per cent. could not be identified with any type. He calls attention to what may be an important point, that F. Griffith's strains were from cases which included many children, and almost all the strains of the other workers were from epidemic cases in the Navy and Army.

Nicolle, Debains and Jouan (1918) investigated the epidemic strains in France and compared them with the ' parameningococci ' of Dopter and representative strains of Gordon and Murray. They divided their series into A, B, C and D, of which A and B included certain of Dopter's strains and all of Gordon and Murray's, while C and D were rare. They used monovalent horse-serum and disregarded the absorption test as unnecessary and complex, but as Dopter (1921) remarks, had they availed themselves of this test, they would, no doubt, have modified their opinion.

The correlation of these various classifications is set out in Table I and it shows a general agreement between results which is not apparent from a cursory examination of the literature. It also shows a confusion of terminology, particularly as regards the American use of ' parameningococcus ', for which there is no justification, and this term should no longer be used in any case.

I would suggest a tentative subdivision of meningococci into four main groups : A, B, C and D, with the, at present known, types A I, A III, B II and B IV. Using this as a basis, further work will clarify the serological classification of meningococci and reveal whether there are additional groups and types, as there very well may be, more especially in sporadic cases.

Opsonic Reaction.

It is difficult to apply this reaction to the meningococcus as laboratory cultures are readily ingested by leucocytes, although freshly isolated spinal strains are resistant to phagocytosis. This test applied to serum has given very variable reactions in the hands of different authors, and definite conclusions cannot be drawn. Kolmer, Toyama and Matsunami (1918) and Hektoen and Tunnicliffe (1921) have shown that the opsonic activity of antimeningococcus serum can be greatly increased by the addition of fresh guinea-pig serum, or, better still, fresh human serum.

Precipitin Reaction.

The meningococcus quite readily stimulates the production of a precipitin serum, and a very good antigen is obtained by centrifuging suspensions of cocci disintegrated by the osmotic pressure method (Murray, 1928). The reaction has been put to very little use and Dopter (1921) says it runs parallel to the agglutination reaction.

Complement Fixation.

There is considerable disagreement in the literature concerning the preparation of the antigen to be used in this test, but it has been shown to have a remarkable selectiveness for the type of coccus, although apparently independent of the agglutinating titre of the serum (Bell, 1920).

TABLE I.

Dopter (Strain in Brackets).	Gordon and Murray.	Pullon.	American (Rockefeller).	F. Griffith (A. S. Griffith).	Nicolle, Debains and Jouan.
Meningococcus (M.T.)	I	C	Parameningococcus	I A	A
	III	A	Irregular meningococcus.	I B	
Parameningococcus α (P.S. and P.W.)	II	B	Normal meningococcus.	II	B
Parameningococcus β (P.L.) ..	IV	—	Irregular meningococcus.	II	
Parameningococcus β (P.M.)	—	—	—	—	C (Rare).
Parameningococcus γ (P. 25)	—	—	—	—	D (Very rare).

Relationship to one another of the agglutinable types of meningococcus described by different authors who included known strains in their series.

Bacteriolytic Reaction.

The lysis of sensitized cocci by alexin *in vitro* is difficult to demonstrate, and when successful, higher dilutions of serum are often more bactericidal than lower ones. This I have noticed frequently with this reaction, using other bacteria besides the meningococcus, and, I think, it is due to complement fixation by the precipitin reaction. Matsunami and Kolmer (1918) believe that the multiplication of the surviving cocci in the medium enriched by the addition of the fresh serum obscures the reaction. At the same time it must be observed that the meningococcus is readily lysed by fresh guinea-pig serum.

Dopter (1910) was able to show an extensive specific lysis of sublethal doses of meningococcus in the peritoneal cavity of guinea-pigs previously treated with antimeningococcus serum. The reaction is somewhat complicated by a marked phagolysis which coincides with the rapid disappearance of the cocci. Dopter (1921) claims that this reaction clearly distinguishes between Nicolle's groups A, B and C. Absorption of the serum prior to its injection results in sharp differentiation of agglutinable types (Dopter and Pauron, 1914).

The intravenous injection of 1 c.cm. of unheated antimeningococcus serum and 1·5 c.cm. of a heavy suspension in saline of a 24-hour culture of meningococcus, results in the rapid death of the guinea-pig (Briot, and Dopter 1910). The reaction is specific and when combined with absorption distinguishes between Nicolle's types A, B, C and D (Dopter, 1921).

Anti-endotoxin.

That no eminently satisfactory demonstration of anti-endotoxin in anti-meningococcus serum has yet been possible is due to the difficulty of working with endotoxin.

Hitchens and Robinson (1916) suspended their cultures in fresh guinea-pig serum diluted 1 : 4 with saline, and it is possible that a number of cocci were thus lysed and their endotoxin liberated. They injected their serum intraperitoneally two hours before giving the cocci by the same route and obtained very consistent results. They showed definitely that a serum which protects strongly against a strain of one agglutinable group will fail to protect against a strain of another agglutinable group (meningococcus and parameningococcus of the American nomenclature). Gordon (1918, 1920) attempted to use liberated endotoxin in his tests, and obtained evidence that anti-endotoxin was selective within the limits of agglutinable types. He injected a mixture of endotoxin and serum which had stood at 37° C. for 30 minutes. Krauss and Doerr (1908) found that anti-meningococcus serum possessed a specific protective power against autolysed suspensions and cultures of meningococcus macerated in 0·1 N NaOH. They observed that greater protection was afforded if the serum was injected 6 to 18 hours before the toxin than if injected half an hour before, and that this in turn gave greater protection than if the

serum and toxin were mixed *in vitro* and half an hour later the mixture injected into the animal. Both Hitchens and Robinson (1916) and Gordon (1920) have shown that the sera which prove effective therapeutically are protective in laboratory tests on mice by their methods.

Therapeutic Sera.

There is no doubt that extraordinarily powerful therapeutic sera are produced from time to time, but all who have tried have experienced inconsistent results without succeeding in tracing the cause of their failure. Until a reliable method of titrating the value of these sera in the laboratory is arrived at there is little likelihood of progress. There are clear indications, however, that a suitable serum is only produced by prolonged immunization. Sera resulting from 12 to 16 months' treatment of the horses were more powerfully protective than those obtained in 3 to 7 months (Hitchens and Robinson, 1916). It also seems to be true that the intravenous route is the most favourable, but the choice of dose, the state of the culture, the intervals between doses and the stage at which to bleed are all in need of further investigation. Every available immunity reaction has been employed as an index of potency of the serum for curative purposes and up to now agreement has not been reached.

It seems to me the most desirable attributes of a curative serum are : (1) Power to neutralize endotoxin ; (2) Power to promote phagocytosis ; (3) Bacteriolytic power. I believe that an efficient serum should be well endowed with all three. Nevertheless, it must be remembered in employing such a serum that the cerebrospinal fluid is deficient in alexin, even when purulent, and in order to render phagocytosis and lysis effective the serum should be activated by the addition of fresh human or fresh guinea-pig serum to it (M'Kenzie and Martin, 1908 ; Fairley and Stewart, 1916 ; Kolmer, Toyama and Matsunami, 1918 ; Hektoen and Tunnicliffe, 1921). The addition of fresh cerebrospinal fluid from normal persons and from cases to antimeningococcus serum does not promote phagocytosis (Davis, 1907). The carrying out of the necessary tests is no easy undertaking, as all who have tried know well, but the adjustment of the conditions so that each of these reactions can be titrated separately and accurately will, I believe, allow of a laboratory estimation of the therapeutic value of a serum.

Vaccines.

The literature of meningococcus vaccines is reviewed by Dopter (1921), by Worster-Drought and Kennedy (1919), and the latter authors add their own experiences. There appears to be a distinct impression that vaccines are of material therapeutic assistance once cases have passed the initial acute stage or when the patient's reaction has abated to result in chronicity. The dosage is variable and no stress is laid on the culture selected nor its condition. The treatment of carriers by vaccines does not appear to have been effective (Colebrook and Tanner, 1916) nor is there

any support for prophylactic vaccination. In view of the possibility of selecting susceptible individuals on the lines of Heist and S. and M. Solis-Cohen (1922) the question of prophylaxis might be reinvestigated with advantage.

Infection in Man.

In the ordinary conception of the process of infection by the meningococcus three factors are invoked to account for the observed facts :

(1) The chance of contact with the infective agent ;

(2) The virulence of the infective agent ;

(3) The susceptibility of the host.

The first of these is determined by the environment, and a number of important facts bearing on it have been accumulated, a few of which will have to be considered. The second almost invariably assumes a potentiality solely invested in the bacterium, with the implication that it is an acquisition independent of any contributory action on the part of the host. The third assumption is required to explain certain observed irregularities or accidents in the course of infection, either of resistance (such as chronic carriers exhibit) or of exhaustion or helplessness (as in the development of meningitis), and is only necessary so long as virulence is regarded as an independent attribute of the parasite alone.

To this conception of infection I am not able to subscribe. To my mind, the interpretation which best fits the observed facts is to regard virulence as the measurable resultant of the antagonistic efforts of both the host and the parasite. This resultant can be made to vary, in actual fact, by influencing the host alone just as much as by influencing the parasite alone (Murray, 1924, 1929). With this view there is no need whatever to employ the conception of susceptibility, nor any of the implications involved in (2) and (3) referred to above. The balance swings for or against the host by virtue of its primary defences, at least in so far as the establishment of infection in the normal host is concerned. By primary defences I mean physiological processes adaptable to meet circumstances, brought about by infection, for which they are not designed, but with which they cope sometimes well, sometimes indifferently. The processes involved are such as phagocytosis, repair, excretion and permeability. The processes of specific immunity responses I regard as the secondary defences of the host, which are only developed by direct stimulation of the host cells or perhaps by mutual physical and chemical modification of existing substances of both host and parasite. The term susceptibility could conveniently be confined now, by definition, to express that a host is unusually lacking in effective co-ordination of its primary defensive processes, or that certain of those processes are deficient ; which means that the host is here contributing to virulence. The secondary defences do not enter into the process of meningococcus infection until late or with treatment, and, as will be shown, may bring about a fatal termination by ill-balanced action.

The Source of Infection and Method of Spread.

Infection is only acquired by association with previously infected persons who themselves are seldom aware of anything being wrong and commonly are unconnected with a case of meningitis. Were it not for extension of the infection, with its grave localizations in occasional cases, it is possible that no attention would have been given to the meningococcus. The variety of hygienic conditions which contribute to the increased incidence of infection are discussed in Volume VI, but it may be mentioned here that overcrowding (Glover, 1918[1 & 2]) and the increased ' spraying capacity ' occasioned by catarrhal conditions of the upper respiratory passages (Eagleton, 1919) are of the greatest importance.

The ' carrier ' is, in fact, the typical and commonest case of the disease, and this term is useful to signify a person with an infected nasopharynx ; either one who has not developed meningitis, &c. (primary carrier), or one who having recovered from the more serious states persists in harbouring the meningococcus in his nasopharynx (secondary carrier). Primary carriers are frequently spoken of as contact or non-contact carriers, according to whether they have been associated with a case of meningitis or not. The carrier rate of contacts is usually higher than of non-contacts, but when these two rates become identical in a selected population it is a warning of danger, and ' cases ' usually occur when the general carrier rate reaches 20 per cent. (Glover, 1918[1 & 2]). For the most part primary carriers remain infected for not more than six weeks, about 10 per cent. of them persist for a longer time and are arbitrarily called ' chronic carriers ', and about 2 per cent. continue to carry for 6 to 12 months or more. Every form of carrier is potentially infective, so it comes about that about 95 per cent. of all cases of cerebrospinal fever have not resulted from previous cases, but from contact with the so-called ' healthy ' carrier.

The Mechanism and Course of Infection.

The meningococcus having gained the nasopharynx, it is possible that its elimination is largely due to phagocytosis and lysis, particularly as Dopter (1921), Albrecht and Gohn (1901), Westenhoeffer (1905, 1906) say there is hyperæmia and a catarrhal condition of the nasopharynx in every case of infection, even though it may be transitory.

The sum of evidence suggests that in the majority of cases of meningitis the onset of symptoms follows very rapidly on the infection of the nasopharynx, and it is only rarely that recognized primary carriers develop meningitis. There is very little evidence of even a slight development of antibodies in chronic carriers ; it is, therefore, upon the primary defences that opposition to the meningococcus must depend, and it is probable that phagocytosis and lysis by ' leukines ' are the principal processes involved. However, it has been shown that virulent strains are able to resist phagocytosis, and, even when ingested, may survive long enough to be liberated again unscathed, and so may well be carried back

by leucocytes and liberated into the tissues and body fluids. But Davis (1905) showed that the meningococcus would not grow in the blood of the normal persons and cases he examined and Heist and S. and M. Solis-Cohen (1922) showed that carrier strains are able to grow in the drawn blood of something less than 5 per cent. of normal men, and they surmise that it is probably among this group that the cases of meningitis occur. Dr. G. D. Heist was himself an extreme case of a person whose blood completely failed to inhibit the growth of carrier strains, and he later died of meningococcus meningitis in spite of early diagnosis and vigorous treatment.

The virulence of the strain may play a part, and it seems possible that the influence of the leucocyte exudate in the nasopharynx, either in the source of infection (carrier or case) or in the case itself, might act in much the same way as leucocyte extracts do *in vitro* and result in raised virulence. Thus, it is possible that in the small percentage of infected persons who develop meningitis there is no need for more than a direct extension into the inflamed submucous tissue to ensure generalization.

To account for meningitis certain authors have supposed a trans-ethmoidal route, or direct extension through the bony walls of the sphenoidal sinus, to be possible, but the weight of evidence favours dissemination of the cocci by the blood-stream, particularly as lesions and conditions other than meningitis have to be accounted for. Richey and Helmbold (1923) review six cases of meningococcus meningitis following head injuries, certain of which were fractures and the remainder bled from the nose or ear.

There is no doubt that the meningococcus shows a predilection for the subarachnoid space. Probably the absence of alexin in the cerebrospinal fluid, the impenetrability of this space to circulating antibodies in the blood, and the interference during infection with its normal drainage by perivascular infiltration with leucocytes, renders it an eminently suitable habitat for this organism.

There is little evidence to support the idea of excretion of the cocci by the choroid plexus, other than the congestion and relatively slight cellular infiltration in acute cases which die early. But petechiæ occur in the subependymal tissue, and these with the general perivascular infiltration and œdema are some of the earliest lesions, and may be the method of infection of the cerebrospinal fluid. Remembering always that petechiæ in the skin can be caused by meningococcal emboli in the smaller arterioles, so even capillary emboli in the subependymal tissue or the meninges would probably be sufficient in a region so susceptible to injury as the central nervous system.

Often the precocity and preponderance of the meningitis so overshadows the pharyngitis and septicæmia which precede it that such cases are regarded as a primary meningitis. Thus, too, localizations in the skin, joints, serous cavities and other sites have been regarded as secondary to the meningitis, though in reality they rank with it as metastases.

The diffuse nature of the meningitis is considered to be against its originating in a single focus, but once the cocci have entered the fluid they would rapidly permeate it, and, aided by its flow, would spread over the whole subarachnoid space in a very short time. This view is supported by Flexner's (1907[2]) experimental meningitis in monkeys.

The end to be desired, whatever the stage of infection, is the destruction of the cocci and the elimination of their products with the least possible damage to the tissues of the host. When the infection is confined to the nasopharynx, phagocytosis, lysis, and bacterial antagonism are probably sufficient to ensure recovery, as the charged cells and products of the cocci are eliminated on an external surface. When, however, the primary defences fail, then the barrier of the mucous membrane is interfered with by congestion, catarrhal lesions, œdema and extravasation of leucocytes which are unable to cope with the invading micro-organisms, the body fluids exhibit no bactericidal power, and so the infection generalizes and metastasizes. At this stage the case shows any of the many possible variations: there may be fulminant septicæmia without meningitis; meningitis as a late development; the local conditions may clear up individually and develop again, &c. Then it is that the effect of the secondary defences come into play, whether they are developed naturally, or introduced therapeutically.

Autoserum therapy and the injection of fresh normal serum probably is effective in certain cases of meningitis by reason of the alexin introduced, and the effect is to increase phagocytosis and lysis. The impermeability of the theca to immune bodies, even when inflamed, makes it imperative to introduce specific serum directly into the subarachnoid space, but without alexin the stimulus to phagocytosis and lysis of which the serum may be capable, is lost. Ineffective phagocytosis with subsequent liberation of large numbers of lysed cocci, or lysis of sensitized cocci on a large scale by alexin, may result in a massive liberation of endotoxin, which is sufficient to prove fatal, unless the therapeutic serum is well endowed with anti-endotoxin.

That this is no idle speculation is demonstrated by Dopter's 'Vein Test' (p. 313), and by the fact that in attempting to titrate the value of antimeningococcus serum in mice, the animals which receive certain samples of antiserum die sooner than the controls, certain of which have received normal serum, while others have received the coccus only. The experiments of Davis (1907) are also significant, in that the subcutaneous injection of killed meningococcus culture was far more toxic to a normal individual than to two cases of meningitis of whom the blood was strongly bactericidal. The degree to which phagocytosis may protect is shown by those experiments in which Dopter (1921) injected culture intravenously, and then after an interval followed it by antiserum, with the result that the cocci were guarded by phagocytosis from immediate lysis. Murray's (1929) experiments with the liberation of endotoxin by leucocyte extracts

show that phagocytosis is not effective unless the lysed products are retained by the cells. Thus, although the cocci present may all be killed by specific treatment and thereby the infection be cured, nevertheless, the patient may die on account of the massive liberation of endotoxin, whether this is brought about by bacteriolysis or by ineffectual phagocytosis.

So, to reiterate my belief, to be effective it is essential that a curative serum should possess opsonic properties, bacteriolytic properties and anti-endotoxin ; all three are required.

Experimental Infection in Animals.

Meningococcus infection seems to be confined to man. The ordinarily used laboratory animals (rabbits, guinea-pigs, rats and mice, horses, goats and sheep) do not allow of the production of characteristic disease as seen in man, not even when subdural inoculation is used. Certain of these animals are readily killed by cultures inoculated intraperitoneally or intravenously, while others are extremely resistant. Care has to be exercised in immunizing animals, because even ordinarily resistant animals may die suddenly very shortly after a repeated dose of culture, possibly because of the massive liberation of endotoxin.

General experience of the smaller animals places the mouse and rat as the most susceptible, next the young guinea-pig, then the adult guinea-pig, while the rabbit is remarkably resistant. This order is confirmed by Matsunami and Kolmer (1918), using their test of the bactericidal activity of the whole blood. With these animals inoculation with a single dose of culture causes death more often by the intraperitoneal route than the intravenous, and subcutaneous inoculation appears not to infect. Von Lingelsheim and Leuchs (1906), Flexner (1907[2]), Macdonald (1908), produced rapidly fatal meningococcal meningitis in monkeys by intrathecal inoculation of cultures. The infection spread rapidly over the whole cerebrospinal system, and there were even localizations at the base of the brain. By using repeated small doses Flexner was able to reproduce a more chronic form of lesion.

Experimental chronic infection with the meningococcus is as difficult to produce in the healthy laboratory animal as it is with most other pathogenic organisms. Death usually occurs within 48 hours, otherwise the animal recovers rapidly and survives.

The Isolation of the Meningococcus from the Nasopharynx.

The finding of a few Gram-negative intracellular cocci in microscopical preparations of the mucopus from the nasopharynx is not of diagnostic significance, as it is common to the meningococcus and a host of morphologically identical cocci. Identification by culture is essential. But in cases, particularly at post-mortem, from which almost pure cultures of meningococcus have been grown from the nasopharynx, the distribution of the Gram-negative cocci has a distinct interest from the point of view

of the method of penetration, and concerning this point direct evidence is still needed, more especially from sections.

The collection of the material from the nasopharynx requires care, as it is most necessary to avoid contamination with saliva, and, to this end, West's swabs are of great use. These are bent glass tubes through which the flexible swab holder is passed and protruded after the tube is in position behind the soft palate. The apparatus is sterilized as a whole, with a wool plug at each end of the tube. In using it there is an advantage in applying an appreciable pressure, and the pharyngeal tonsil and any adenoid tissue should be particularly brushed over. The mucus containing meningococci is often clear and pearly in appearance, and is tenacious, sticking to the swab easily.

The cultural conditions already described must be carefully observed, and the plates must not be discarded until after 48 hours' incubation at least, when characteristic colonies are sought for and subcultured. With practice these are quickly recognized, but at first it is a laborious process and many colonies are picked off which are not meningococci. It is possible that J. Gordon and McLeod's (1928) use of the indophenol reaction might prove of advantage here, in the way they have found it useful for the recognition of gonococcus colonies.

Many of the Gram-negative cocci commonly present in the nasopharynx form colonies which cannot be confused with those of the meningococcus, but others are indistinguishable, and, until they have been isolated in pure culture and examined more closely, they must be regarded as suspect. Certain of these cocci have been described (von Lingelsheim, 1906, 1908; Elser and Huntoon, 1909), but many of them have not, and they have been roughly grouped for convenience of expression as 'pharyngococci' by certain authors. G. S. Wilson and Smith (1928) and S. P. Wilson (1928) have investigated the characters and differentiation of certain of these cocci. It is not out of place here to give a brief description of the more important of them.

Micrococcus flavus I (von Lingelsheim, 1908), which appears to be identical with the chromogenic Group II of Elser and Huntoon (1909), very closely resembles the meningococcus, not only in its colony, but in other cultural characters and in delicacy. According to v. Lingelsheim it ferments lævulose, but Elser and Huntoon say that it does not. Its distinguishing character is the production of a clear pale yellow pigment in the primary culture after 24 hours growth; but it may lose this property in subculture. What appears to be this organism has frequently been described as agglutinating with normal rabbit serum, and it does not absorb the agglutinin from any type meningococcus serum. This has been described as 'flavus agglutination', by many British authors, who quite incorrectly refer to the organism as Flavus III of von Lingelsheim. In point of fact there is no danger of confusing Flavus II and Flavus III of von Lingelsheim or Chromogenic groups I and III of Elser and Huntoon with the meningococcus because they produce a distinct yellow pigment.

Micrococcus catarrhalis (Seifert, Pfeiffer), ought not to cause difficulty, as, compared with the meningococcus, it produces a thicker, more opaque and whitish colony, with a steep and often irregular margin, and it emulsifies badly in water or saline ; no sugars are attacked by it.

A number of organisms have been isolated from the nasopharynx which are culturally indistinguishable from the meningococcus. Though they did not agglutinate with nor absorb the type serums of the 1915 epidemic, which then accounted for 98 per cent. of the spinal strains of meningococcus, certain of them agglutinated with Flexner's polyvalent serum (Fildes and Baker, 1918). It would be difficult to say that some of these were not meningococci, and, no doubt, at the present time in Britain they would be accounted so, for very many of the spinal strains from the sporadic cases now occurring do not agree with any of the serological types of the 1915 epidemic.

Glover (1918) compared the meningococcus carrier rate with the carrier rate due to these inagglutinable strains, and found that at the time when there were no cases those men with agglutinable meningococci formed only a small proportion of the total carriers ($\frac{1}{6}$th to $\frac{1}{20}$th), but with the occurrence of cases those with agglutinable cocci increased to $\frac{2}{3}$, $\frac{3}{4}$ and more of the total carriers of organisms resembling meningococci.

Even though it may be that these organisms are not of importance and do not cause disease, they do occasion a great deal of work and worry when it is necessary to detect carriers, since they can only be recognized by serological tests. For this reason, and also for the more important reason that there is evidence (Hitchens and Robinson, 1916 ; M. H. Gordon, 1920) that curative serum is most effective when it corresponds to the agglutinable type of coccus infecting the patient, it will be well to include in this chapter a short description of certain modified methods which have been used as short cuts in serological identification of meningococcus types.

However useful these short cuts may be, the warning must be heeded that there is no justification for using them for any purpose other than as a guide. The results they give must be checked by agglutination and absorption tests using a standardized technique, with full controls, applied with all available precautions against variation.

In the ' rapid method ' of Nicolle, Debains and Jouan (1918) 1 c.cm. of a suspension in 1 per cent. saline of the first pure culture obtained, is placed in each of a number of tubes (the number being determined by the number of agglutinating sera, plus one for normal serum) and to each tube is added 0·5 c.cm. of serum. The tubes are then tightly plugged with non-absorbent wool, and the contents run gently backwards and forwards from end to end of the tube for 3 to 5 minutes, during which agglutination will occur in the tube containing the corresponding type serum. It is very important to use 1 per cent. NaCl in this method.

Fildes and Baker (1918), Fildes (1920) absorbed a polyvalent serum with the strain they wished to test and then by using type cultures

determined which type ' agglutinin ' had been removed. This is probably the most comprehensive method yet devised, but it depends entirely on both the strains used for the test suspensions and the serum being sufficiently representative. When this method is applied carefully it is capable of clear-cut results, and under the conditions now prevailing in Britain it might easily prove very useful. Krumwiede, Cooper and Provost (1925, p. 72) criticize the method and define its limitations. It might be much improved by using a mixture of suitable monovalent sera.

Bell (1920) applied the well-known method of mixing equal parts of a thick suspension of cocci and serum on a slide, with good results. He found it necessary to absorb the ' group agglutinin ' from the high titre sera he used, and so produced highly specific type-sera.

In America the practice seems to be to divide all meningococci into three groups: normal meningococcus, ' parameningococcus ' and irregular meningococcus. Group sera are produced by immunizing horses with as many spinal strains of the group as possible and the cultures are assigned to a group according to which of these sera agglutinate it.

Such tests as these may serve where the sole aim is diagnosis.

Outline for Laboratory Diagnosis.

Morphology. The meningococcus is frankly Gram-negative. There is always a considerable variation in the depth to which individual cocci take stains, and this combined with irregularity in size, even in members of the same colony, are its most characteristic morphological features, and are both most marked in cultures. The cocci are often associated in pairs, more particularly in inflammatory exudates. In these the cocci are frequently intracellular, but such a condition is not diagnostic, as it is shown also by the other Gram-negative cocci found in man. There appears to be a decided relation between the number of intracellular cocci and virulence; certainly there are more extracellular cocci in the more fulminant cases.

The presence of a Gram-negative coccus in the cerebrospinal fluid may be considered diagnostic of meningococcus infection, although other allied organisms have been described from this situation, particularly after fracture of the skull. With the exception of *Flavus I* (von Lingelsheim) these other cocci are easily differentiated in culture. Gram-negative cocci in the blood and nasopharyngeal mucus can only be recognized by their cultural and immunity reactions.

Cultural characters. Growth is not obtained on ordinary media unless fresh body fluids are added. Agar media smeared with a little fresh blood or serum or ascitic fluid are suitable as an expedient. On such media growth occurs more readily in primary culture than on sub-culture. Dorset's egg is the most suitable medium for maintaining cultures, as they will survive on it for many months. On Murray's ELD agar (pH 7·2) profuse growth is obtained without the addition of body fluids, and, with an adjustment of the reaction to pH 7·8 or 8, the same applies

to the gonococcus. This medium has the additional advantage of maintaining virulence. Other suitable media for growth are MM and formol-serum-agar used by Nicolle, Debains and Jouan (1918) and Fildes' serum agar.

The optimal temperature for growth is 37° C. Chilling and drying of the material to be cultured reduces the frequency of successful culture, and material received by post is commonly sterile. It is often an advantage to warm the medium to 37° C. before inoculating it. When cocci are scarce in the cerebrospinal fluid their numbers can be considerably increased by incubating the fluid at 37° before culturing it, and there is often an advantage in using the centrifuged deposit.

Swabs from the nasopharynx should be plated immediately and the plates should be kept warm during transit to the laboratory. The most satisfactory results are obtained when the nasopharyngeal mucus is heavily inoculated on to a restricted portion of the plate, which is then incubated for a few hours before the material is spread over the remaining surface of the medium.

Contamination with saliva is to be avoided on account of the inhibitory action of certain common salivary organisms. The advantage of West's swab lies in the ease with which salivary contamination is avoided by its use.

On suitable media the colonies are characteristic and with practice they are easily recognized. They are transparent, lenticular, smooth, moist and the margin is entire. Under a low power of a microscope the outer zone is perfectly clear and has the appearance of a ' halo '. On turning from the window to a wall the colonies assume a homogeneous pearly grey colour without iridescence. A thin blue colony or a ' stippled ' colony may be ignored.

The sugar reactions on single colony cultures are most satisfactory on agar media which give profuse growth, but it is advisable not to add too much serum or other body fluids. The capacity of the meningococcus to produce alkaline substances when cultures are freely oxygenated must be borne in mind. The sugar reactions cannot invariably be relied on, as occasional strains behave in an irregular way.

It is possible that the indophenolase test of J. Gordon and McLeod (1928), Ellingworth, McLeod and Gordon (1929) might greatly facilitate the recognition of meningococcus colonies in cultures from the nasopharynx.

Serological reactions. The final recognition of the meningococcus depends upon the agglutination test, but as this is complicated by agglutinable types its application is not always easy. In the face of an established epidemic routine absorption tests using monotypical sera prove the most satisfactory. At other times the application of Fildes' method, using a polyvalent serum, preferably a mixture of monovalent sera, and a number of representative strains offers certain advantages. The recognition of the meningococcus from sources other than the cerebrospinal fluid requires experience, and for the application of serological tests tried sera and strains are essential.

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CHAPTER VI. THE INFLUENZA GROUP OF BACTERIA.

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Definition.

This group, of which *B. influenzæ* (Pfeiffer) is the most important member, is limited to those bacteria which, unlike all other known bacterial species, require for permanent cultivation on artificial media accessory substances present in blood and fresh vegetable tissues. All the members of the group are aerobic, non-motile, Gram-negative bacilli varying in length from 'coccal' forms, in which the longest diameter may be as small as $0 \cdot 2\mu$, to threads of 20μ or more; in thickness they may vary up to $0 \cdot 8\mu$.

Of the accessory substances or 'growth factors' on which their permanent cultivation absolutely depends, two have been distinguished and named X and V. The X-factor is associated with hæmatin and with less well-defined substances present in fruits, potato and other vegetable material; these latter, like hæmatin, all contain iron and possess particular activity as 'peroxidases', accelerating the transfer of oxygen to guaiaconic acid or benzidine from peroxides as in the guaiac and benzidine tests for blood. The V-factor, present in fresh animal or vegetable tissue including blood, fresh potato, yeast and many bacterial cultures, resembles in its physical and chemical behaviour the antiscorbutic vitamin C; it is thermolabile but not readily adsorbed, being in these respects the reverse of the X-factor.

Classification.

According to the accessory substances which they require, three divisions may be distinguished in this group of bacteria: A, those which require both X- and V-factors, e.g. the *B. influenzæ* (Pfeiffer) and the *B. koch-weeks*, which is probably a synonym; B, those which can elaborate V-factor for themselves in a nutritive medium, but require the X-factor to be supplied to them in the form of hæmatin or (heated) vegetable tissue, e.g. *B. hæmoglobinophilus canis* (Friedberger), the only known representative; and C, those which do not require the X-factor but require the V-factor to be supplied to them, e.g. the non-hæmolytic *B. para-influenzæ* (Rivers) and the hæmolytic 'X' bacilli of Pritchett and Stillman.

This classification, suggested by Fildes (1924) and, with some modification, by Valentine and Rivers (1927), is primarily correlative; it

includes in one group along with the influenza bacillus, species which, though differing otherwise, have in common an absolute and inalienable dependence on one or both of the growth-factors described. Intermediate strains occur ; for example, there are strains which belong to class A in their growth-requirements, but, on their other characters (hæmolysis, &c.), are obviously much nearer to the species found in class C ; and other strains can be found indistinguishable in every respect from *B. influenzæ* (Pfeiffer) except that, in their demands upon accessory factors, they belong to class C and not to class A. The existence of intermediate strains points strongly to the advantage of associating these different species in one group.

As regards nomenclature, reference should be made to the chapter by Andrewes on bacterial classification in general (Vol. I), but the term ' influenza group ' will be used in the course of the succeeding discussion as a convenient colloquial expression for this collection of species. Objection may be taken to the term on the ground that it prejudges the unsettled question whether Pfeiffer's bacillus is the true cause of influenza. But no such prejudice is implied ; Pfeiffer's bacillus will almost certainly retain the specific name, *B. influenzæ*, since it was found originally in influenza and has persistent association with this disease, even though it may some day be found not to play the primary and exclusive part in causation.

The alternative title, the ' hæmophilic group ', has several disadvantages ; there is, in the first place, the possibility of growing the bacilli in presence of vegetable tissue instead of blood ; and secondly, the fact that some members of the group are independent not only of hæmatin but of its vegetable counterpart. Furthermore, it has the unfortunate implication that it might include those species the growth of which is favoured by blood in the medium, though their dependence on it is, in fact, neither absolute nor permanent. The committee on classification of the Society of American Bacteriologists have fallen into this error in including under the generic name ' Hemophilus ' bacteria such as *B. pertussis*, *B. duplex* (Morax-Axenfeld) and *B. ducrey*. These bacteria have obviously different growth requirements from those of *B. influenzæ*, and the others associated in the opening definition, and to give them the same generic title can only lead to confusion.

The Influenza Bacillus.

Historical.

Bacteriological investigation of influenza began with the great pandemic of 1889–92, the first outbreak of the kind for nearly forty years. No significant observations were published, however, till January, 1892, when R. Pfeiffer (1892) announced the discovery of the influenza bacillus, a minute bacillus present in enormous numbers in the sputum of cases of the disease. The discoverer, working in the Institute for Infectious

Diseases in Berlin, had seen the bacilli in the sputum in the spring of 1890, and a photograph taken at the time was published by Kirchner ; but Pfeiffer's actual work on influenza did not begin till November, 1891. In his first announcement he stated these bacilli could not be subcultivated, though they produced an abundance of very small colonies on agar plates smeared with the sputum. In May, 1892, he was able to announce that he had overcome the difficulty of subculture ; the influenza bacillus required hæmoglobin for growth in artificial culture media. In 1893 Pfeiffer published the complete account of his work on influenza, in which he stated the reasons for regarding the bacillus as the ætiological agent, and gave a full account of the methods for isolating it and maintaining it in pure culture. Other observers, among whom were Canon and Kitasato, had undoubtedly seen and suspected the minute bacilli in influenza sputum, but their attempts to identify it with the cultures they obtained on nutrient media not containing blood were recognized as failures. Pfeiffer's discovery of the hæmoglobinophilic nature of the minute bacilli in influenza sputum received widespread confirmation during the short remaining period of the pandemic. Wassermann, in 1893, went so far as to say that their presence was invariable in influenza : ' Wo Influenza, da Influenza-bacillen '. But during the succeeding years when influenza existed, if at all, in the form of localized outbreaks of feverish catarrh, doubts began to be expressed. On the one hand, many observers had difficulty in finding influenza bacilli in such outbreaks, and, on the other hand, influenza bacilli were found by others in diseases such as measles, whooping cough, tuberculosis, &c., in which clinical influenza was absent. As regards these findings, negative and positive, it must be admitted that, with regard to the former, the degree of skill and care required may not have been applied, and, with regard to the second, that the presence in the air passages of other hæmoglobinophilic bacteria and bacteria only temporarily so, such as *B. pertussis*, may have given rise to confusion.

Nevertheless, it had to be accepted that bacilli indistinguishable from the influenza bacillus of Pfeiffer were to be found not only in health, but also, perhaps to an abnormal extent, in diseases in which clinical influenza was not present, while on the other hand, outbreaks of clinical influenza occurred in which Pfeiffer's bacillus could not be found.

It is not surprising that an explanation of these facts was felt desirable and that to some bacteriologists the simplest conclusions were : (1) that Pfeiffer's bacillus was not the actual cause of influenza ; and (2) that, since causal bacteria could not be found, a filterable virus was the most probable agent. In the absence of epidemic influenza this supposition could not be subjected to experimental examination ; it remained purely hypothetical, though the experiments of Kruse (1914) and Foster (1916), which seemed to show that a filtrate of the nasal secretions in common colds could produce similar catarrh in normal persons, were regarded by some as supporting evidence.

During the pandemic of 1918–20 the two theories were in sharp opposition. While many bacteriologists were satisfied from their observations on the disease, both in the living and *post mortem*, that Pfeiffer's view of the ætiology should be maintained, others were impressed by the same difficulties as before, the inability to find the bacillus in cases of influenza and its presence in apparent health. The latter sought for the causal agent in filtrates of the diseased secretions. Their failure to demonstrate a filterable virus will be discussed in detail below, but it must be admitted, on the other hand, that there remain many points in the theory of Pfeiffer's bacillus as the cause of influenza which do not admit of a simple explanation. There has been obtained, however, by the work of the last few years a much greater precision in the methods of culture and identification of the influenza bacillus. The analysis of the ' growth factors ' required by the different members of the influenza group (Olsen, 1920 ; Fildes, 1920, 1921, 1924 ; Thjötta and Avery, 1921 ; Rivers and Poole, 1921 ; Rivers, 1922) has especially advanced our knowledge and increased the general scientific interest of these bacteria.

Morphology and Staining Properties.

In smears of the sputum of influenzal catarrh the influenza bacilli appear characteristically as minute cocco-bacilli, measuring 0·2 to 0·8μ in their longest diameter and often arranged in dense masses ; they are Gram-negative and stain rather faintly with the usual dyes, dilute carbol-fuchsine giving perhaps the best result. A preliminary treatment with dilute acetic acid improves the staining of the bacteria in sputum films. The bacilli are often in pairs end-to-end and may show bipolar staining. In culture they stain rather more easily, and three morphological varieties may be distinguished (Levinthal and Fernbach, 1922) ; (*a*) the cocco-bacilli, the commonest variety ; (*b*) the rod-forms in which the bacilli appear as straight slender rods with rounded ends, the length being three or four times greater than the breadth : curved, almost comma-like, forms also occur in this group : strains of the rod variety may develop long slender filaments in which the division into bacilli, if present, is hard to perceive ; (*c*) the pleomorphic group in which different shapes and grades of size are present, along with irregularly-shaped and ' monstrous ' forms ; in this group the formation of long swollen threads is particularly common, but such swollen threads may be found in cultures of the other two groups as well. The same strain may appear in successive cultures as belonging to different groups ; formerly cocco-bacillary, it may become rod-like or pleomorphic and again revert to its original microscopical appearance (but these changes are not by any means frequent). The pleomorphic phase is found especially, but not exclusively, in cultures which have grown in a medium poor in the essential growth factors ; it may also be produced by growth in a medium containing 3 per cent. salt (Davis, 1907) or 2 per cent. sodium citrate (Preuss, 1921). The long slender filaments described

in connection with the rod variety are not associated with poor nutrition but appear most commonly in colonies which have undergone ' rough ' transformation.

In the present state of knowledge it is difficult to prescribe upper limits of size for influenza bacilli. Some of the strains found in the normal nasopharynx and in such conditions as ' influenzal ' arthritis and meningitis, though behaving like typical influenza in their growth requirements, are definitely larger than the strains commonly present in such abundance in influenzal sputum or lungs. Such large strains do not always retain their abnormal size in subculture, and, though some specific difference may eventually be found, must be regarded, at present, as influenza bacilli. It may, perhaps, be said with safety that a culture in which the bacilli do not exceed $0 \cdot 5\mu$ in thickness (apart from pleomorphic swollen individuals) is a ' microscopically typical ' influenza bacillus, but that larger bacilli are to be regarded as doubtful until all the means of distinguishing them otherwise have been exhausted.

It is generally agreed that the influenza bacillus is non-motile and does not possess flagella, but no thorough investigation of the matter appears to have been made, and in Kristensen's (1922) opinion it is not impossible that motile strains may occur.

Cultivation.

The difficulties experienced by the early workers have been entirely overcome by recent methods of cultivation. The original blood-smeared agar of Pfeiffer gave only minute colonies ; ' dewdrops ' barely visible to the naked eye, though, by the use of pigeon's blood, which liberates a more abundant supply of the ' peroxidase ' substance (vide *infra*), rather more vigorous growth was obtainable [Czaplewski (1902) describes the technique required]. Still better growth was found by Voges (1894) to occur when blood was mixed with agar at 100° C. (blood put in Petri-dish and flooded with agar straight from the steamer). Grassberger (1898) observed that influenza bacilli on a blood plate in the neighbourhood of a staphylococcus colony appeared as ' giant colonies ', and blood media containing staphylococci and their products, the cocci having been killed if necessary by a short heating, were used with success by Allen (1910), Knorr (1924) and many others. The term ' satellitism ' is also employed by French writers for this phenomenon (Meunier, 1898). A great advance in technique was made by Levinthal's (1918) discovery that blood boiled for a few moments in nutrient agar gave an optimal medium for the influenza bacillus. This heating method had, in fact, been discovered by Cohen and Fitzgerald in 1910, but had been generally forgotten. Matthews (1918) described almost simultaneously a medium of equal excellence prepared by subjecting blood to tryptic digestion and mixing the product with ordinary nutrient media. Fleming (1919) showed that treatment of blood with normal HCl followed by neutralization also transformed it into a favourable state for inducing influenzal growth. Fildes (1920) showed that

peptic digestion of blood gave a sterile stable translucent mixture of the growth factors necessary and his technique may be said to give the maximum regularity and efficiency in cultivation.

By the use of these media some of the special requirements formerly attributed to the influenza bacillus (Olsen, 1920) disappear (for methods of preparation, vide Vol. IX). It is no longer necessary to use agar in a proportion not higher than 1 per cent. ; the reaction of the medium may vary from pH 6·8 to 8·0 instead of being restricted as in ordinary blood agar to pH 7·3 to 7·5 ; temperatures from 22 to 42° C. will maintain growth instead of 25 to 38° C : growth will occur on well-dried agar instead of on a surface specially kept moist, a useful property in making cultures from contaminated sputum ; cultures may safely be kept for 10 instead of 3 to 5 days.

Unlike those of many bacteria, cultures of the influenza bacillus cannot be kept alive with certainty for long periods in the refrigerator. For prolonged growth, culture in meat-water can be recommended : heavily inoculated and kept at 37° C. such cultures remain alive for at least a month. Ungermann's leptospira medium has also been used with success for keeping purposes.

The influenza bacillus is strictly aerobic : in liquid media, even optimal, one must employ shallow layers to get the most copious growth. Fildes (1921) has shown that the short-lived growth of the bacillus which occurs on his medium under anaerobic conditions depends on oxygen in the medium, and has suggested that such oxygen is perhaps not merely dissolved but in loose combination. On ordinary blood agar (smeared or mixed), the colonies of the influenza bacillus barely reach a size visible to the naked eye : under the microscope they show no structure, but appear as clear ' water-droplets '. On the optimal media, on the other hand, colonies of 3 mm. diameter are easily obtainable, and they may reach even greater dimensions if growth is allowed to continue to its maximum (about 48 hours at 37° C.). Such colonies are circular, translucent, colourless, flat discs with a sharply contoured edge and very fine granulation in the centre. In consistency the growth resembles thin oil paint and is neither watery nor sticky. On plates inoculated from sputum the colonies are highly characteristic and easily distinguishable from the bacterial inhabitants of the mouth and nasopharynx. Meningococcal colonies have some resemblance in shape and size but are always much more opaque. The influenza strains already referred to as consisting of bacilli of abnormal size, found both in the nasopharynx in health and in the cerebrospinal or joint fluid in cases of meningitis and arthritis, have very similar colonies but are often distinguishable by a bluish iridescence in oblique light and a slightly greater opacity.

Under unknown conditions, but especially in old laboratory cultures, a ' rough ' transformation occurs, resembling, in general, the ' rough ' change among Salmonellas, &c. The condition appears in degree varying from a slight irregularity of the surface with slight increase in coherence

to extreme wrinkling of the surface and serration of the edge of the colony, which then consists of granular friable material or sometimes of a tough membrane detachable *en masse*, and impossible to emulsify. It is probable that early stages of this rough transformation are present in most artificial cultures of the influenza bacillus, since emulsification in normal saline is followed by precipitation, especially on heating to 60° C., in the case of many strains. When they are visibly rough such ' auto-agglutination ' occurs even in distilled water (author's observations).

The Accessory Growth Factors.

Historical.

The growth-stimulating action of blood and the additional stimulus leading to ' giant colony ' formation which is conferred on the medium by the growth in it of saprophytic bacteria were historically the first indications that two factors were necessary for optimum growth of influenza bacilli. Ghon and Preyss (1902, 1904) in a controversy with Cantani (1901, 1902) proved that hæmoglobin or hæmatin must be present for this stimulating action of bacteria to occur. Davis (1917) pointed out that a vitamin substance was required in addition to hæmoglobin, and that this additional substance could be obtained not only from other bacteria, but from fresh plant or animal tissue. Sterile slices of vegetables placed on blood agar produced ' giant ' growths of influenza colonies as ' satellites ' around them. Davis compared the two factors to the vitamins A and B. Thjötta and Avery (1921[1]) showed that two distinct and separable substances are present in blood, neither of which is sufficient alone to maintain the growth of influenza bacilli. One which they called the X-substance is associated with hæmoglobin, is heat-stable and acts in minute amounts : the other, the V-substance (vitamin-like), can be extracted from red cells, from yeast and from vegetable tissue, and is relatively heat-labile. Fildes (1921) discovered independently the separate nature of the two factors in blood. In the preparation of his peptic digest (vide Vol. IX), the hæmatin occasionally precipitated out completely, the mixture having probably been left too acid. This precipitate, washed and dissolved in soda, when added to nutrient agar, failed to promote growth. The supernatant fluid, which contained extremely small quantities of blood pigment, similarly failed, but when precipitate and supernatant were combined copious growth resulted. Thjötta and Avery (1921[2]) showed that the factors were both present in vegetable tissue and that, especially, sterile potato could maintain growth indefinitely in liquid media. They were the first to establish the fact that growth is not absolutely dependent on blood pigment. Rivers and Poole (1921) should also be mentioned : they showed that a convenient way of displaying the two factors was to take filtered yeast extract as the V-factor and autoclaved blood as X. Kent (1923) confirmed the findings of Thjötta and Avery as to both factors being present in fresh vegetable tissue.

[It is interesting to note that Cantani (1901) found that acid peptic digestion of blood gave a product which rendered agar an admirable influenza medium, but he reported that it soon became inert, while Shiga, Imai and Eguchi (1913) found that potato extract agar along with blood produced a medium on which influenza bacilli (as well as *B. pertussis*) gave a thick growth. Since the reasons for these facts were not at the time clear, it is perhaps natural that they lapsed into oblivion.]

Among German workers, Olsen (1920[2]) suggested that the globin fraction of hæmoglobin corresponded in its activity to the ' giant colony ' action of saprophytic bacteria, while the hæmatin fraction had some ' peroxidase ' function which enabled the influenza bacillus to obtain its required oxygen (see later).

Kollath (1924, 1925), Kollath and Leichtentritt (1925), Knorr (1925), Knorr and Gehlen (1925[2]) all confirmed the findings of Thjötta and Avery and made further observations on the nature of the growth factors.

In the French literature, Agulhon and Legroux (1918), and Legroux and Mesnard (1920) should be mentioned. They found that a filterable dialysable saline extract of red cells (prepared at 80° C.) had a vitamin-like action in the culture of influenza bacilli. The active principles were sensitive to heat and especially to alkalis ; the authors called them ' hormones de croissance ' ; they did not recognize the double nature of the factors involved.

Experimental.

In the analysis of the part played by growth factors in the cultivation of bacteria, the experimental details are of great importance. The addition of large culture masses in inoculation may permit growth in an unsuitable medium, either owing to the transference with the bacteria of portions of the original medium, or owing to the fact that the dead bodies of the transferred bacteria may provide utilizable food material. Hence it is necessary to employ the smallest inoculum possible and, in many cases, to wash the bacteria in salt solution before inoculation. In liquid media, especially, the utilization of the dead bacilli as food material by their descendants may permit of as many as four or five transferences of viable bacteria in a medium which is, in fact, quite incapable of furnishing all the food factors necessary. The neglect of this phenomenon, to which Kollath (1924) has given the name ' cannibalism ', has been the cause of many of the contradictory statements to be found in the literature of influenza culture. On the other hand, one must make certain that the inoculum is not too small in proportion to the bulk of medium inoculated ; it is well known that many bacterial species inoculated in minute amount in a medium quite capable of maintaining their growth may fail to develop. It is probable, in fact, that all bacteria require some substance—growth factor or enzyme—to be present in sufficient concentration before they can start to metabolize their food material, though in the actual process of metabolism they produce such substances in plenty. A control in an optimal medium is, thus, necessary for these minimal inocula.

Another point the neglect of which has led to much confusion, is the extremely small amount of blood pigment which is necessary for growth in a medium in which the other conditions are well fulfilled. Body fluids, organ extracts and even chemical derivatives of organic origin may contain sufficient traces for some degree of growth. To take a recent example, Jacoby and Frankenthal (1921) described influenzal growth as occurring when histidine chloride was added to nutrient agar, and drew far-reaching conclusions as to the importance of tryptophane derivatives in growth. But Knorr and Gehlen (1925[1]) showed that the histidine gave a positive benzidine test for blood pigment, contained, therefore, the X-factor, and, with the help of saprophytic bacteria, sufficed, if poorly, for the growth of influenza bacilli.

Another consideration is that different strains of influenza bacilli seem to have different capacities for storing up the essential growth factors. Some exceptional strains will permit a series of passages, though not indefinitely, on a medium on which the usual influenza strains entirely fail. Hence it is always advisable to employ several strains in experimental work on growth conditions.

The V-Factor.

Sources. As has been indicated the substances on which this factor depends can be elaborated by practically all bacteria except classes A and C of the influenza group. With many of them the amount produced is small, and can be demonstrated only by the ' giant ' influenzal growth which appears in the neighbourhood of a living colony. With others sufficient may be produced for cultures killed by moderate heat to show the effect when mixed with medium containing only the X-factor. Extracts of such cultures in watery alcohol also contain the V-factor. Yeast, as shown by Thjötta and Avery (1921), is particularly rich, and watery extracts of it, even after removal of all its growth products by washing (Fildes, 1923), show a strong growth-stimulating action.

Potato (Kollath, 1924) is also a rich source of V-factor, and tomatoes, lemons, apples, bananas, coco-nuts, green peas and similar products have all been shown to contain more or less abundant supplies. Animal organic matter also contains it when in the fresh condition : the red blood corpuscles contain more than the serum.

Physical and chemical characters. The V-factor is sensitive to heat : autoclaving at 120° C. destroys it in all media : at lower temperatures, 100° C. and below, destruction is a function of time and probably of oxidation : it depends also very much on the reaction of the medium, the V-factor being much more resistent in acid (pH 4·5) than in alkaline surroundings. Its extreme sensitiveness to alkali is well shown in the preparation of Fildes's peptic digest, which is ruined by alkali above pH 7·5. It is also destroyed by drying, oxidation being here again probably the actual cause. It is capable of passing through filters without serious loss, i.e. it is not readily adsorbed, though some loss occurs in

contact with bone charcoal (Thjötta and Avery, 1921[1]). It is capable of dialysis through parchment and of diffusing in such colloids as agar (cf. the satellite phenomenon). No systematic examination of its behaviour towards physical agencies appears, however, to have been made, so that precise figures are lacking. Chemically, potent V-factor extracts may contain little available nitrogen, i.e. they are not nutritive in the sense of providing constructive material. Weichardt and Riedmüller (1925) describe biuret-free extracts from animal organs in watery alcohol as potent growth stimulants (along with X-factor) for influenza bacilli. It appears certain that V-factor, from some sources at least, is soluble in watery alcohol. The fact that it can be precipitated by alcohol along with the cells from blood or bacterial cultures (Agulhon and Legroux, 1918; Kalkbrenner, 1921) does not negative this.

The destructive action of fresh serum on V-factor is important as it accounts for some of the formerly unexplained observations in the culture of influenza bacilli on media containing blood. Davis (1921[1]) noted that fresh serum inhibited the 'giant colony' effect (satellite phenomenon) of bacterial cultures: Terada (1922) described a ferment-like action of the serum on blood corpuscles, destroying their growth-promoting action for influenza bacilli. Knorr (1924) made a special study of this effect. Incubation for 3 hours of laked blood, as compared with unlaked, rendered the former useless for the preparation of Levinthal agar; the V-factor liberated by laking from the red cells had been exposed to the destructive action of the serum while that in the intact red cells escaped. The sera of different animal species varied in the degree of this destructive activity which they possessed. Sheep, rabbit, guinea-pig, horse, rat, man and pigeon serum are given by Knorr as a series of diminishing activity in this respect. The V-factor of lemon juice, like that produced by bacteria and that present in red cells, could be similarly inactivated (complete destruction in 4 hours at 37° C.) in contact with fresh serum. The destructive power of the serum could be removed by heating at 56° C., one of the reasons for the superiority as a medium of boiled blood as compared with fresh blood-agar.

Comparison with vitamin C. In its origin and in its behaviour towards physical agencies the V-factor certainly bears a close resemblance to vitamin C. The latter, like the V-factor, is found especially in fresh plant tissue; it dissolves in water and alcohol, dialyses through collodion membranes and can be passed through a Berkefeld filter without loss of potency. It is not readily adsorbed, but loses activity in contact with bone charcoal. It is more or less quickly destroyed by heat at 100° C. or higher, destruction being much more rapid in an alkaline than in an acid medium; it is particularly susceptible to drying and oxidation. Davidsohn (1924) says the bacterial V-factor is more resistent to heat and oxidation than the anti-scorbutic vitamin, but the differences cannot be great.

On the other hand, it is obvious that assumption of identity is quite unjustifiable, since, in each case, the active agent may be only one ingredient

of the complex mixture in which both are available. One significant fact against their being identical is that yeast, which is so potent a source of V-factor, has no anti-scorbutic activity (M.R.C. Committee on Vitamins, 1924), and it would in any case seem unlikely that animal and bacterial metabolism should depend to such an extent on an identical substance. Experiments on animals have shown that bacterial cultures known to be good producers of V-factor have no anti-scorbutic action in the diet (Kollath and Leichtentritt, 1925), though, like yeast, they may act as anti-neuritics in vitamin B deficiency. The same authors (1925) tested the blood of scorbutic guinea-pigs and found, as it seemed, that its growth-promoting power for influenza bacilli had much diminished but could be restored by feeding the animals with lemon juice or, *in vitro*, by adding V-factor from potato. Similar lack of V-factor was shown by the blood of a child suffering from Barlow's disease. But, when the red cells of scorbutic guinea-pigs were tested separately, it was found that their content in V-factor was unimpaired : the diminished growth-promoting power for influenza bacilli of the whole blood was due to a great increase in the destructive action of the serum on V-factor. The same authors showed incidentally that B-vitamin starvation and also simple inanition led to no diminution in the V-factor of the blood : this had already been shown by Thjötta (1924).

Possible mode of action. There are singularly few speculations in the literature as to the possible mechanism of V-factor in aiding influenzal growth. The suggestion of Jordan Lloyd (1916) that the accessory substance may act as a catalyst accelerating the splitting of nitrogenous substances is perhaps the most important. Another possibility is that it is essentially an organic peroxide, the function of which is to provide a supply of immediately available oxygen to the bacteria (cf. Bacterial Respiration, Vol. I, and Burnet, 1927).

The X-Factor.

Sources. Hæmoglobin from any species of animal, with a few doubtful exceptions (Davis, 1907), is the principal source of the X-factor. The work of many investigators—that of Olsen (1920) and of Fildes (1921) may be specially mentioned—has confirmed Pfeiffer's original statement that it is the iron-containing fraction of the pigment in which the activity resides. Hence hæmatin is the simplest pigment to display full activity, whereas hæmatoporphyrin, its iron-free derivative, and bilirubin, chlorophyll and pyrrol, its chemical neighbours, are quite devoid of action. Hæmoglobin itself is comparatively feeble, or perhaps even quite impotent, so long as it remains intact. Hæmocyanin, the blood pigment of crustaceans, in which copper takes the place of iron, contains no X-factor (Davis, 1907, *et al.*). Other iron-containing compounds, inorganic or organic, even the inorganic peroxidase prepared by the mixture of potassium ferrocyanide and ferrous sulphate, have none of this hæmatin effect. But vegetable tissue, especially potato, also contains X-factor,

i.e. a substance which in combination with V-factor of whatever origin renders a nutritive medium suitable for permanent cultivation of influenza bacilli.

There has been much doubt whether bacteria can produce X-factor. Neisser (1903) was able to carry on influenza bacilli through 20 subcultures on ordinary nutrient agar in symbiosis with *B. xerosis*, symbiosis, in this instance, meaning that the colonies were actually mixtures of the two bacteria : it does not appear that satellite ' giant ' colonies occurred. Similar results have been obtained by many others, but in most cases, if not all, the requisite precautions to exclude X-factor present in the medium or carried along with the inoculum were not taken. Davis (1921[1]) has, however, confirmed Neisser's work, also with *B. xerosis*, and Kollath (1925) gives experiments which plainly show that colloidal iron (ammoniated oxide of iron), added to agar containing V-factor only (potato extract), or even only glucose as an addition, enabled an air coccus and *B. fæcalis alcaligenes* to produce symbiotic colonies in which influenza bacilli grew abundantly (but exclusively in the mixed colonies). Recently Kollath (1926) has shown that other iron compounds (e.g. potassium ferrocyanide) may be ' activated ' by ultra-violet rays so as to give a similar X-factor effect in symbiosis. This activation was associated with acquirement of peroxidase activity by the iron salt. Hence it has probably to be admitted that under certain conditions (especially with a suitable iron-compound properly distributed in the medium) X-factor can be elaborated by certain bacteria. Valentine and Rivers (1927) have shown that *B. hæmoglobinophilus canis*, which is absolutely dependent on X-factor for its growth, can be grown in symbiosis (liquid medium) with bacteria of class C (hæmolytic X-bacilli and *B. parainfluenzæ*) which are independent of X, but give a positive peroxidase reaction in mass and may thus be presumed to prepare X-factor themselves. Further work on this subject is required.

Physical and chemical characters. The X-factor is conspicuously heat-resistent ; it withstands autoclaving at 120° C. without serious loss, especially if in the form of hæmatin : potato, on the other hand, is slightly but distinctly weaker in X-factor activity after autoclaving. It is readily adsorbed by finely divided matter and can be completely removed from Levinthal's medium, for example, by shaking up with bone charcoal (Knorr, 1924). It is associated with a ' peroxidase ' action (Olsen, 1920 ; Fildes, 1921), i.e. material containing X-factor has the power of accelerating the transfer of oxygen from a peroxide, e.g. H_2O_2, to guaiaconic acid (producing the blue pigment of the guaiac test for blood) or to benzidine ; in extreme dilution such material may no longer show this ' peroxidase ' activity and yet still possess some action as X-factor in influenzal growth. Autoclaved potato, and vegetable tissue generally, is devoid of ' peroxidase ' activity, though still sufficiently active as X-factor : this may simply mean that influenzal growth is a more delicate indicator than the ' peroxidase ' test. The X-factor associated with hæmatin is diffusible in

agar ; that in vegetable tissue is apparently not, but is fixed to the vegetable cell, hence the impossibility of preparing an agar medium suitable for influenza bacilli using potato extract alone. As has been mentioned, iron must be present in the blood pigment to enable it to act as X-factor : the same probably is true of the factor in vegetable tissue, since all such material contains iron, but in default of a soluble preparation proof is lacking.

Comparison with vitamin. None of the dietetic vitamins in animal nutrition are comparable with the X-factor : probably a similar substance is a constant product of animal metabolism, but is indispensable to animal life and hence always present. In plant nutrition, however, the phenomena of iron-starvation may partly depend on the absence of some such factor.

Mode of action. Fildes (1921, 1922) was the first to correlate the 'peroxidase' activity of both blood and vegetable X-factor with the hypothesis that its action was of a similar catalytic nature, accelerating the transfer of oxygen from peroxides in the medium or from the atmosphere to the bacillus. This theory has received general support (Knorr, 1924 ; Kollath, 1924, *et al.*). It depends chiefly on the coincidence of the two activities, a coincidence which is perfect, with some explainable exceptions. Fildes (1921) has explained the feeble X-factor action of hæmoglobin, which is an active 'peroxidase', by showing that it takes up the liberated oxygen itself, and will even reduce the oxidized (blue) guaiaconic acid. (He regards the deteriorative effect of excess of fresh blood in an influenza medium as partly due to this action ; but the serum effect on V-factor also comes in). The alternative hypothesis, originally put forward by R. Pfeiffer himself (1893), that iron is an essential component of the influenza bacillus, as in the case of *Crenothrix polyspora*, and, therefore, must be present in its nutritive media, is faced by the difficulty that media containing abundant iron not in the particular combination of hæmatin cannot maintain influenzal growth.

If the 'peroxidase' theory is correct, the respiration of influenza bacilli is quite a special case among bacteria (vide Bacterial Respiration, Vol. I). They would appear to be unable to utilize atmospheric oxygen directly, and be able to employ the intermediate peroxides only with the help of a catalyst which in normal circumstances they can find nowhere but in blood. If this is so, they are obviously obligatory parasites. In the mucus of the human respiratory tract, however, there is, probably quite often, a sufficient amount of blood pigment. Fichtner (1904) long ago showed that heated influenzal sputum, not blood-stained, added to agar made an optimal medium for influenza bacilli, and I can confirm him for the three specimens I have examined for this point.

Demonstration of the Separate Factors.

The methods of demonstrating the separate existence of the two factors have been indicated in the course of the preceding discussion but may conveniently be recapitulated here. The presence of V-factor of

any source may be demonstrated by the use of a nutrient medium, liquid or solid, containing autoclaved hæmatin (vide Vol. IX) ; a proportion equivalent to 1 in 1,000 whole blood is a convenient amount. Such a medium will not grow influenza bacilli except on the addition of: (1) living bacteria (staphylococci, &c.) or an extract of a bacterial mass killed by heat at 60° C., (2) extract of yeast (1 per cent.) (vide Vol. IX), (3) extract of potato or other vegetable matter (filtered or centrifuged clear) ; the equivalent of 5 per cent. of the tissue should be added for copious growth, (4) fresh serum or organ extract (free from blood pigment).

The presence of X-factor may be demonstrated by the use of a nutrient medium containing any of the above products but free from any trace of blood pigment : contact with animal charcoal will remove the X-factor of blood. In such a medium growth of influenza bacilli will fail, but it will be copious on addition of blood-pigment (1 in 1,000 or even greater dilution) or (heated) portions of vegetable tissue.

For testing the growth requirements of individual cultures under examination the use of autoclaved hæmatin agar plus a staphylococcus inoculation will be found most convenient. On such agar *B. influenzæ* grows only in the neighbourhood of the staphylococcus growth ; *B. hæmoglobinophilus canis* grows diffusely but fails on hæmatin-free agar, while the hæmolytic ' X ' bacilli and others of class C will grow in the neighbourhood of the staphylococcus growth on hæmatin-free agar as well.

Dependence on Peptones.

Growth of the influenza bacillus is not dependent on artificially prepared ' peptones ', since, as has been shown by Thjötta and Avery (1921[2]), it can be carried on indefinitely in a solution of phosphates (buffered) or, as shown by Knorr and Gehlen (1925[2]), in ordinary sodium chloride solution to which (in each case) pieces of sterile raw potato have been added ; no doubt the proteins and carbohydrates of the potato furnish the requisite elements for building up the bacterial protein. But it is curious that this statement applies only to growth in liquid media containing pieces of potato. Hæmatin with potato water alone made up to a solid medium with water agar will not give growth and even the addition of peptone, of some brands at least, will not remove the difficulty. There must, therefore, be a third requirement (besides the accessory substances, in themselves not nutritive) in the artificial growth of influenza bacilli. This third factor is probably of an amino-acid nature ; further work is necessary to determine it more precisely.

Biochemical Activity.

Action on proteins. The influenza bacillus has no proteolytic action and liquefies neither serum nor gelatin. But it has a curious power of forming acid in protein-containing media which is even greater than that of many more saprophytic bacteria [Kristensen (1922) on growth in coli-fermented broth]. This has to be taken into account in carbohydrate fermentation tests.

Indole-formation is of particular interest. The power of forming indole divides influenza bacilli into two sharply divided groups; one group consists of strains which form indole more or less abundantly (in tryptic digest broth containing the requisite growth factors), while the strains in the other group form none. (The test is carried out with Ehrlich's reagent in the usual way; Witte's peptone containing little free tryptophane is least active as an indole source, tryptic digest most.) The fact of indole-production by influenza bacilli was first discovered by Rhein (1919), followed by Jordan (1919), Rivers (1919–20) and Yabe (1921). All are agreed on the sharp distinction between positive and negative indole-formers. The possible taxonomic significance of the groups will be discussed under serology but it may be said here that, unlike other bacterial groups in which indole-formation is definitely a specific difference, the influenza group is not divisible into species on the strength of this reaction.

Action on carbohydrates. Kristensen (1922) in a reasoned argument came to the conclusion that 'no certain and even moderately marked power of fermentation could be demonstrated either on solid or in liquid media' for influenza bacilli. He pointed out that the acidity which can be observed in glucose agar or glucose broth after growth of the influenza bacillus is no greater than that produced in the same medium without glucose, the medium in both cases having first been rendered as nearly as possible sugar-free by coli fermentation. The same applies to other fermentable carbohydrates and absolves from the necessity of reproducing here the varying results recorded by Levinthal (1918), Stillman and Bourne (1920), Rivers and Kohn (1921) *inter alios*. Rivers and Leuschner (1921) state that *B. hæmoglobinophilus canis* is a strong fermenter and Kristensen agrees with this (9 strains tested).

Nitrate reduction. Rivers (1920) and Rivers and Kohn (1921) have studied this particularly. They find that all influenza bacilli reduce nitrates to nitrites.

Hæmolysin. The power of dissolving red cells is practically confined to one class, Class C, of the influenza group, q.v.

Toxin-production. This question will be discussed under pathogenic action. The toxic filtrate which can be obtained from influenza cultures resembles in its action the similar filtrate that can be obtained from the cholera vibrio, the paratyphoid group, the colon bacillus and many others. It differs from the toxalbumins of diphtheria, tetanus, &c., in not being quantitatively neutralizable by antiserum and appears to belong to the group of poisons which act directly on the endothelial cells.

Resistance to External Influences.

There are few recent studies of this question. Pfeiffer (1893) noted that *B. influenzæ* cultures emulsified in tap water were dead in 32 hours at room temperature, and that they were particularly susceptible to drying. Fromme (1918) found that the influenza bacilli could no longer be recovered

from influenza sputum dried for 5 hours. Onorato (1902) made extensive experiments on the effect of sunlight, drying, heat, cold and chemicals on cultures. His results show that direct sunlight kills in 3 to 4 hours, drying *in vacuo* in $\frac{1}{4}$ to $\frac{1}{2}$ hour, drying in air in $2\frac{1}{2}$ hours, steam at 100° C. in 5 to 60 seconds, at 62° C. in 2 minutes, at 55° C. in 20 minutes, at 45° C. in 30 minutes; freezing at −15 to 20° C. in 2 hours; almost instantaneous death was produced by the usual disinfectants (2 per cent. phenol, &c.). Fromme (1918) noted, as an exception, that 3 per cent. hydrogen peroxide failed to kill; this observation requires confirmation; it may depend on the presence of catalase in the medium which would rapidly inactivate this disinfectant. Kristensen (1922) found quite definite differences in the heat-resisting capacity of different strains. At 50·5° C. certain 'atypical' strains survived half an hour, whereas many strains were killed in the same time at 47·5° C.; he found, contrary to Onorato, that the bacilli would stand freezing for 24 hours, and that there were distinct differences in different strains in their resistance to drying for 5 hours, but that, when kept dry, all were dead in 24 hours at 37° C.

Serological Reactions.

The principal fact in the serology is the diversity of the antigenic components. Antigenic identity of large percentages of the strains, such as creates the types of pneumococci or salmonellas, does not exist in the case of the influenza bacillus. This is the conclusion arrived at by all those who have made extensive observations on the agglutination reactions of influenza strains.

Agglutinin in Human Patients.

Much of the work on the development of agglutinin in the human patient has been gravely affected by this diversity, though, by using several different strains, positive reactions have been found in a high proportion of patients (Levinthal, 1918 *et al.*). Malone's (1920) observations may be taken as an example, and are of special interest in that he was able to test agglutinin formation in individual patients with the strain isolated from each. He found that 32 out of 39 such cases developed agglutinin against the homologous bacillus, whereas, when heterologous strains were used (a mixture of four), only 22 out of 52 patients' sera reacted. He never found a negative reaction with the homologous strain when the serum gave a positive reaction with heterologous mixture, and the titre was always lower for the latter than for the former. Fleming (1919), Wollstein (1919), Wilson (1919), Hartley (1919), Edington (1920), have all found that different strains give different results as to development of agglutinin in convalescent influenza patients.

The titres observed by Malone (1920) are fairly representative. He used emulsions in distilled water from cultures on pigeon's blood-agar and incubated the mixtures with serum for 2 hours at 50° C. He found that the maximum titre appeared during the third and fourth weeks after

the onset of the illness, and that it varied in different patients from 1 in 32 to 1 in 256 for the homologous strain and from 1 in 8 to 1 in 64 for the heterologous mixture : the majority of the titres in each case lay in the lower ranges. His control sera, 25 in number, were all negative except 2 ; one of these had suffered from lobar pneumonia and the other from ankylostomiasis. Malone found that six months after recovery from influenza no agglutinin was demonstrable. Wilson's (1919) observations are interesting and are confirmed by Edington (1920). He found that the agglutination of Pfeiffer's bacillus in severe influenzal broncho-pneumonia was strong, as high as 1 in 1,000, during the febrile period, but disappeared on defervescence and was absent in those who had been afebrile for from 6 to 32 days. Normal persons and late convalescents gave no agglutination, even in dilution of 1 in 2. Edington's cases, in whom he made the same observations, apparently independently, occurred in a small outbreak among soldiers in the spring of 1920 in which influenza bacilli were isolated in 30 out of 31 examined. Sharp and Jordan (1924) would explain agglutination of this kind as due to an increase of normal non-specific agglutinin in the blood ; they observed it also in the acute stage of measles.

Agglutinin in Animals.

The antigenic diversity of strains of the influenza bacillus can be shown much more clearly by the preparation of agglutinating sera in animals (especially rabbits).

Technique. No peculiarities in technique have been noted as necessary. Rabbits receive intravenously living cultures from surface growth suspended in salt solution ; doses of 5 to 80 mgm. of moist growth may safely be given, and, after a sufficient number of such injections, spread over 2 to 3 months, titres of 800 to 2,000 are usual. Emulsions in salt solution of surface growth containing 2 mgm. per c.cm. will be found suitable for test, such emulsions being mixed with an equal volume of the diluted serum. With a good many strains half-normal salt solution or even distilled water may be necessary in order to avoid confusion with the salt agglutination which occurs in higher strengths of saline. I agree with Kristensen (1922) who reports that old cultures form more stable suspensions than younger ones : I found also that well-dried agar was more suitable than fresh agar for this purpose. Incubation of the mixture should be performed for 4 hours at 50 to 55° C., with half-immersion of the column of fluid. The type of agglutination is that characteristic of non-flagellate bacteria, i.e. fine somewhat granular clumps, but these never become so firm as with dysentery bacilli and they can usually be dispersed by shaking. Tests for absorption of agglutinin are best performed by emulsifying moist growth scraped from agar in the diluted serum. According to the titre 4 to 8 mgm. of such growth will remove all the agglutinin from 1 c.cm. of 1 in 50 dilution of the homologous serum : contact at room temperature is sufficient and the maximum absorption is reached almost instantaneously (though a very slight increase can be obtained by

longer contact). As with meningococci, repeated absorptions may increase the amount of agglutinin removed in the case of heterologous strains (author's observations).

Serological Groups.

Kristensen (1922) and Scott (1922) give the results of such serological examination, using large series of strains of different origin, and have come independently to very similar conclusions. [These conclusions agree generally with the findings of many other workers in this field, for example those of Valentine and Cooper (1919), Povitzky and Denny (1921), Seligmann and Wolff (1920), Bieling and Joseph (1920), Maitland and Cameron (1921).] Kristensen (1922) used 14 monovalent (rabbit) sera to test 127 strains, and Scott (1922), 12 monovalent sera to test 275 strains, the majority of the strains of both authors being either from cases of influenza and pneumonia or from healthy persons. Kristensen's conclusion is that ' as far as can be seen, there are innumerable continuous gradations, so that there is no basis for a division into serological subgroups ', though he found that the strains isolated from a distinct group of persons (soldiers in the Jaegersborg camp) had similar serological characters. He noted that a strain might agglutinate (to some extent) with several of his 14 sera and regarded the occasional occurrence of strains serologically identical as ' the exception which proves the rule '. Strains belonging to the same type (i.e. agglutinating strongly with the same serum and absorbing some agglutinin) gave the same agglutination spectrum, i.e. the complete picture of the agglutination reactions with the 14 sera was the same. The writer (Scott, 1922) has also recorded the multiplicity of the antigenic components of influenza bacilli and the fact that several antigens might be identified in a single strain. In the paper referred to I have suggested, on the basis of agglutination reactions, that each strain has a dominant component which may be recognized in another strain, either as dominant, the rarer case, or as subsidiary, the dominant antigen in the second strain being different, but recognizable in a similar way in still other strains. Such relationships can be demonstrated also by absorption of agglutinin ; a strain containing a subsidiary antigen, recognizable from the presence of its corresponding agglutinin in the agglutinin-complex of a monovalent serum, will rob that serum of agglutinating power for other strains the agglutination of which depends also on possession of that particular subsidiary antigen. I found certain antigens to be of common occurrence among influenza bacilli while others appeared to be relatively rare. Taking three of these commoner antigens as recognizable attributes of certain influenza strains, I found that strains containing one or more of these were present slightly, but distinctly, oftener in pneumonia (lobar and influenzal) and bronchitis than in normal persons (two-thirds as compared with half), but could find no sure indication of ' epidemic types ' such as were demonstrable to some extent among meningococci during the prevalence of cerebrospinal fever in 1915 and 1916.

Bieling (1920) speaks of polyvalent and monovalent influenza strains which are probably equivalent to my commoner and rarer antigenic components. He, too, finds several serological types among the polyvalent strains. Maitland and Cameron (1921) speak of the variation in the antigenic value of strains; some had a very narrow range, their serum agglutinating only the homologous strain, while others produced sera having a more catholic action.

Stability of Serological Characters.

Stability of serological characters is apparently as pronounced (in artificial culture) as in other bacterial species. Kristensen (1922) found some variability in the heterologous agglutination whereas the homologous kept very constant. Later (1924) he described variations in meningitis strains revealed by selection of colonies: the new strains were deficient in some of the antigenic components of the original. A similar degradation was observed by Griffith (1918) among meningococci. Park and Cooper (1921) found no change in agglutination behaviour, either on prolonged cultivation or after animal passage. [Lubinski (1924), on the other hand, found much variation in agglutination reaction of strains during subculture]. But whether this stability also applies in the normal habitat, the human nasopharynx, is an open question. The multiplicity of serological varieties is rather in favour of lability in this situation. Records of direct observation on this point are scanty. Valentine and Cooper (1919) and Povitzky and Denny (1921) usually found the same type in repeated examinations of individuals, but occasionally found a change. Kristensen (1925) compared the spinal strain with the strain isolated from the blood or nasopharynx in three cases of meningitis and in each instance found identity. Maitland and Cameron (1921) state that two or more serological races may be present in the same patient. Anderson and Schultz (1921) found no less than five different strains in different situations in one infected person [spinal fluid (meningitis), blood, nose, throat and nasopharynx]. The difficulties in identification hamper work of this kind: it may, nevertheless, be of prime importance in the epidemiology of infections with influenza bacilli [cf. Griffith's (1928) work on the lability of pneumococcus types].

Serological Grouping in Relation to other Distinguishing Characters of Individual Strains.

Yabe (1921) Kristensen (1922) and Scott (1922) all found that the sharp distinction provided by the power to form indole has no counterpart in serology. Indole-formers and non-indole-formers may be identical in antigenic characters. This is remarkable and perhaps unique, since, among other bacterial species, the rule is that indole-formation is a specific difference confirmable by serological methods. The same absence of correlation exists in connection with the morphological varieties (Maitland and Cameron, 1921, and many others), the variations in virulence and

toxicity (Yabe, 1921), and the origin of the strains (catarrh, normal meningitis, conjunctivitis, &c.) (Lubinski, 1924).

Complement Fixation.

Complement-fixation experiments have not added anything material to the serological analysis of influenza bacilli. Bieling and Joseph (1920) found that their high-potency anti-influenza horse serum gave complement fixation in minute doses, not only with the actual strains used in immunization but with others. Kristensen (1922), however, using monovalent rabbit sera found that there was no complement-fixing antibody common to all strains, and that the lack of homogeneity, though not so marked as with agglutination reactions, was sufficient to prevent any classification within the group. A similar difference in the results with horse and rabbit serum is commonly observed in the serological analysis of other bacterial species, e.g. meningococcus.

Other Immunity Reactions.

Bacteriotropin and bacteriolysin for influenza bacilli were both demonstrated by Bieling and Joseph (1920) in their high-potency horse serum. They found that the bacteriotropin titre ran parallel with the titre found in complement-fixation tests and considered that it represented the true anti-infective power of the serum, whereas the agglutination titre reached its maximum early in the treated horse and was independent of antibacterial titre. This, of course, does not prove that the antibody concerned in agglutination is a different substance from that on which bacteriolysis depends (vide Immunity, Vol. VI).

Pathogenic Action.

On Inoculation into Animals.

With pure cultures. No animal except man acquires influenza under ' natural ' conditions ; during the height of influenza epidemics domestic animals and even the quadrumana, exposed as in Zoological Gardens to abundant chances of infection, escape (though recently Mouquet, 1926, saw an influenza-like disease in chimpanzees which the lower apes under similar conditions did not contract). It may eventually turn out that this depends on the inability of the bacillus to grow ordinarily in animals other than man : most workers, including Pfeiffer himself, agree that, in fact, the influenza bacillus in pure culture cannot be made to infect experimental animals. Large doses may be injected into the blood-stream or peritoneum without fatal results, and, in the instances in which death is produced, it is generally found that the bacilli have disappeared (but vide *infra* the experiments of Blake and Cecil). Such pathogenic action in animals, as Pfeiffer (1893) was the first to state, must be due to poisons contained within the bacteria.

This endotoxic action has been the one most studied (Delius and Kolle, 1897, *et al.*), but the results are not of particular interest ; they

resemble, in general, those obtained with the cholera vibrio, the typhoid bacillus or the meningococcus, to take instances in which the ætiological relationship of the bacteria is not doubted, but in which the pathogenic action in animals, as in the case of the influenza bacillus, is different in the main from the disease in man. These results have been got, for the most part, with culture masses from the surface of agar. More recently, however, experiments of greater interest have been made with cultures in liquid media. Parker (1919), Ferry and Houghton (1919), Wollstein (1919) and McIntosh (1922) describe very similar results. Here are McIntosh's findings. Working with strains freshly isolated during the 1918–19 epidemic he found great variations in pathogenicity; some strains were practically without action on animals; three only out of a large number were highly virulent, 2 to 3 c.cm. of broth culture inoculated intraperitoneally killing guinea-pigs regularly in 24 to 48 hours. The symptoms suggested the action of a toxin, and, in fact, bacteria-free filtrates of young (24-hour) broth cultures reproduced them on intravenous injection into rabbits and guinea-pigs. Rather large doses—5 to 6 c.cm. for rabbits and 2 to 3 c.cm. (occasionally as little as 0·5 c.cm.) for guinea-pigs—were necessary to cause death with such filtrates; [Wollstein (1919) found 2 to 3 c.cm. of filtrate from 7 out of 25 cultures tested would kill rabbits of 1,500 gm. in 2½ hours]. Death might be immediate or occur after some hours, and, in the latter case, lung lesions were conspicuous; there were areas of collapse and emphysema with, microscopically, catarrh, exudation of lymph and capillary hæmorrhages, recalling the changes in human broncho-pneumonia; the suprarenals generally showed deep congestion and might be hæmorrhagic.

But the antitoxin prepared by treating rabbits with these filtrates had very little neutralizing power; this observation suggests that the main toxicity was perhaps due to some poison not of the nature of a toxalbumin; [Parker (1919) discusses the question]. It is to be noted, also, that subcutaneous injection of the filtrate was harmless.

The results, in fact, resemble closely those recently described by Theobald Smith and Little (1927) in calves receiving intravenously filtrates of broth cultures of the 'mucoid' races of *B. coli*. The investigation of poisons of this character is a matter of great interest and importance, though it must be remembered that little is known as to their production in the living animal; they may possibly be products of growth only in artificial peptone mixtures. But their peculiar toxic action for endothelium certainly recalls the tendency to capillary hæmorrhage and to endothelial exudate and catarrh which is characteristic of the lesions in clinical influenza.

Methods of inoculation more closely resembling the 'natural' modes of transference of influenza have been more successful. Stillman (1924) made mice inhale finely sprayed emulsions of influenza bacilli; no disease resulted even in animals poisoned with alcohol, but he found that influenza bacilli persisted in the lungs for several days and entered the blood-stream.

The writer had similar results (unpublished) ; a mouse passage strain of influenza bacilli could be found in the nasopharynx and lung of sprayed mice for two days but not longer.

The most important observations on the production of disease in animals by *B. influenzæ* are those of Blake and Cecil (1920). By using a strain the virulence of which had been raised by peritoneal passage in succession through 11 mice and 13 monkeys (cf. Kikuchi, 1909), and by employing the more ' natural ' route of infection, instillation or swabbing of the mouth and nose with cultures or animal exudates infected with this strain, they produced in monkeys (capuchins and *Macacus syrichtus*), an acute rhinitis which spread down the trachea and was followed in most instances by bronchiolitis and broncho-pneumonia ; the illness, though not fatal, bore a close resemblance in its course and complications to human influenza and the lung lesions were identical in morbid anatomy with those seen in fatal influenzal broncho-pneumonia in man (Cecil and Blake, 1920). Influenza bacilli could usually be recovered from the trachea, bronchi and lung, in some cases in great abundance, in others scantily ; they disappeared rapidly on recovery.

A point of interest is that, in the cases where the infection resulted from the simple swabbing or instillation of the nose with culture, the normal bacteria of the nasopharynx (*Streptococcus viridans*, *Staphylococcus albus*, &c.), also spread down the trachea into the bronchi and lung, whereas, when infection was produced by intratracheal injection (needle puncture in the neck), pure cultures of influenza bacilli were obtained from the lesions. The analogy with the bacteriology of the lung in human influenzal pneumonia with its rarer pure influenza bacilli and commoner mixed infection is to be remarked.

These experiments prove beyond doubt that the influenza bacillus may be highly pathogenic to monkeys and may produce in them a morbid process indistinguishable from severe forms of human influenza. They leave unsettled the question of epidemic spread : from this point of view it is regrettable that Blake and Cecil did not attempt to transmit their artificial disease either by transference of the nasal discharge of sick animals to healthy or by simple cage contact experiments. Success with such experiments would have completed the resemblance to the epidemic disease.

It was noted that the virulence of the strain employed rapidly disappeared on subculture in artificial media. This may account for the general failure of previous workers to produce disease with cultures of the influenza bacillus. There may also be required a special adaptation for the species of experimental animal, such as may be presumed to have occurred in Blake and Cecil's peritoneal passage experiments ; this special quality may normally be absent from the bacilli in human sputum.

Intra-ocular infection is of some special interest. Jaensch and Kollath (1925) found that small doses of influenza bacilli—500 bacilli—introduced into the vitreous humour of rabbits produced a severe panophthalmia in

which the bacilli multiplied freely. In all other sites of injection they perish, or at least do not increase in number, even when producing inflammation as the result of their liberated toxins. These results are to be correlated with the (rare) cases of panophthalmia seen in man after cataract operation, in which pure cultures of influenza bacilli have been found.

Pathogenic action on inoculation with mixed cultures. As has been stated above, influenza bacillus septicæmia cannot be produced in animals by injection of a pure culture (with rare exceptions) ; when, however, pneumococci or streptococci are added to the inoculum, a general blood infection can be regularly produced. Jacobson (1901) was the first to record this observation ; he injected dead pneumococci and streptococci along with living influenza bacilli into mice, and grew abundant influenza colonies from the blood and spleen. Kamen (1901) found the same with living streptococci. Similar observations were made during the last pandemic by Wolf (1920) and have been confirmed during recent years by many workers in the course of routine examination of pneumonic sputum ; mice inoculated with such sputum frequently show not only pneumococci, but great numbers of influenza bacilli in their blood. Inoculation along with diphtheria bacilli does not have this effect (Wolf, 1920). The converse action was seen by Huntoon and Hannum (1919) in experiments on mice in which the injection of influenza ' toxin ' (autolysed bacilli) by itself sometimes set up spontaneous pneumococcal or streptococcal infections. Yanagisawa (1919) found that influenza bacilli increased the lethal action on mice of pneumococci and streptococci whereas dysentery bacilli did not.

In explanation of the phenomenon it has been suggested (Kamen, 1901, *inter alios*) either that the cocci act as symbiotic adjuvants for the influenza bacillus in the blood-stream, as they do in artificial culture media, or that, as the result of the coccal infection, the normal defences against invasion of the blood are diminished. There is good evidence (Abrahams, *et al.*, 1917) that such invasion often occurs in man as a terminal phenomenon (though influenza bacilli are rarely found in the blood during life), in cases of mixed pneumococcal and streptococcal infections with influenza bacilli in the lung. The increased invasive power of the combination in animals may be of the same nature as the increased severity of mixed infections in man.

Pathogenic Action on Introduction into Human Beings.

With pure cultures. A fair number of experiments of this kind have been recorded ; Lister and Taylor (1919), Rosenau (1919), Wahl, White and Lyall (1919), Schmidt and Jentsch (1922) and Ishiwara (1923) all failed to produce a definite influenza syndrome in man inoculated in the nose and throat with pure cultures of influenza bacilli ; there is evidence, however, that the subjects might have been abnormally resistant, though two of those in Lister and Taylor's series took influenza when sprayed with fresh untreated influenza sputum. Wahl, White and Lyall found

that some of those inoculated in the throat (not those in the nose) became carriers of influenza bacilli for weeks though not showing symptoms.

Duval and Harris (1919) noted that the injection of 1,000 millions of chloroform-killed influenza bacilli produced in 30 per cent. of those subcutaneously injected symptoms resembling influenza, but subsiding in 6 to 12 hours.

Cecil and Steffen (1921), using freshly isolated cultures of Pfeiffer's bacillus, produced, by intranasal inoculation in adults, varying degrees of local disturbance and general illness. In two, especially, who had received peritoneal exudate from a rhesus monkey killed by a massive dose of influenza bacilli intraperitoneally, definite symptoms of influenza resulted, and the bacilli persisted in the throat for some weeks. In one case, a relapse occurred with purulent nasal discharge, which contained a different serological type from that inoculated. In the case of one known carrier thus inoculated, the bacilli rapidly disappeared (after a mild illness) and the carrier strain (serologically different) alone survived.

Park and Cooper (1921) record 4 cases accidentally inoculated with cultures ; one developed influenza 4 days, and one, 14 days later. The other two did not develop symptoms, and one of them did not even become a carrier, though the other three did.

Pathogenic action in natural infections. Leaving aside for the moment its possible participation in the lesions of epidemic influenza, a long list of situations can be cited in which the influenza bacillus has produced acute or chronic pyogenic inflammation. Nasal sinusitis, otitis media, panophthalmia, conjunctivitis, meningitis, tonsillitis, endocarditis, empyema, appendicitis, cholecystitis, pyelitis, urethritis, pyosalpinx, arthritis, osteomyelitis, subcutaneous and deep abscesses have all been described by various pathologists as yielding either pure cultures of Pfeiffer's bacillus or a mixture of bacteria in which it greatly predominated (references in Ritchie, 1910 ; Lubinski, 1924). Some of the observations may, no doubt, be discounted owing to defects in identification ; in others, especially the meningitis and arthritis cases, there is reason to believe (vide *infra*, p. 382) that special races of the influenza group and perhaps bacteria less restricted in cultural conditions than any actually in the group are more commonly found. But the general fact is definitely established ; the influenza bacillus is one of the pyogenic bacteria.

In the case of inflammation of the bronchi and lung in man, assessment of the importance of the influenza bacillus must be approached with more caution. Some might go so far as to say that it is a mere saprophyte adapted to life in the secretions of the respiratory tract, and multiplying vigorously in them when they are excessive in amount or abnormal in quality. All will admit that, in recent years at least, it is common and abundant in the sputum in simple bronchitis and broncho-pneumonia. On the other hand, in the severer forms of bronchitis and broncho-pneumonia, it is hard to accept the conclusion that the influenza bacillus is innocuous. Take, for example, the ' purulent bronchitis ' which was

prevalent during the winter of 1916–17 among British soldiers in France (Hammond, Rolland and Shore, 1917), at Aldershot (Abrahams, Hallows, Eyre and French, 1917), and elsewhere, though ' influenza ' at the time showed no more than the ordinary seasonal endemic rate. All these observers agreed in regarding the influenza bacillus as the cause, the majority of the cases showing it in great abundance. In some cases it was present in pure culture with no other bacteria to account for the purulent exudate ; in other cases pneumococci or streptococci were also present. (In the Aldershot report, it is noted that the pneumococci found in the sputum in the early stages were of low virulence, but in the pus at death were of full virulence, and the suggestion is made that the association with the influenza bacillus is responsible for this rise of virulence.) In both reports stress is laid on the severe toxic effects seen in the lung tissue surrounding the pus-filled bronchi, i.e. in proximity to the masses of influenza bacilli, and it seems impossible to reconcile such a condition with the mere multiplication in mucus of a bacillus to which the host is indifferent.

On the other hand, severe forms of bronchitis and broncho-pneumonia may be produced by other bacteria ; pneumococcal forms are undoubtedly to be found and, in the outbreak of ' interstitial pneumonia ' which occurred in the winter of 1917–18 in the United States Army camps in America, a hæmolytic streptococcus was responsible. In this outbreak, which began as an increasingly common complication of measles, but later spread independently, MacCallum (1919) found hæmolytic streptococci in the sputum in every case ; in the lung lesions at autopsy they were visible in 30 out of 38, while influenza bacilli were found in not more than 25 per cent. (though in a few they were present in such numbers as to suggest they might have been the active pathogenic agent). In their morbid anatomy MacCallum regarded the cases as practically indistinguishable from the ' purulent bronchitis ' of the British Armies—which he accepted as being due to the influenza bacillus—except that, in the American outbreak, there was always an associated sero-fibrinous or sero-purulent pleurisy.

MacCallum's outbreak is parallelled by an almost precisely similar one described by Macdonald, Ritchie, Fox and White (1918) and by Eyre and Lowe (1918) among New Zealand troops on Salisbury Plain. There were 418 cases of measles and rubella, all complicated by lung inflammation (7 per cent. mortality), and many showing the typical ' purulent bronchitis ' with sero-fibrinous pleurisy. Streptococci (not all hæmolytic) were found in 96 per cent. of the 40 sputa and 12 lungs examined ; influenza bacilli in 72·5 per cent. of the sputa and, in pure culture, in 2 of the lungs. The outbreak occurred in the first three months of 1918, and did not spread outside the camp. Eyre and Lowe record quite similar findings ; 12 out of 14 cases showed influenza bacilli in the sputum, but, in general, hæmolytic streptococci and *M. catarrhalis* were the predominating bacteria.

In the case of pandemic influenza, though not necessarily accepting the influenza bacillus as the primary agent, one may be convinced of its high pathogenicity for man. The intimate association of the bacilli with lesions such as developed during the second and third waves of the great autumn and winter pandemic of 1918–19, is a fact too striking to be explained on any other basis than direct causation.

A full description of the morbid anatomy of these lesions in the great pandemic does not come within the province of this article [vide Studies of Influenza in . . . British Armies (1919), French (1920) and Kuczynski and Wolff (1921)]. It resembles, in the main, that of the ' purulent bronchitis ' and ' interstitial pneumonia ' just referred to. The lesions affect especially, of course, the respiratory organs. Beginning with rhinitis—epistaxis was very common—the inflammation goes on to tracheitis with a peculiar stratification of the epithelium, the ' glazed mucosa ', following on catarrh. Bronchitis and bronchiolitis are produced by a further extension, with secretion of muco-pus or pure pus, and the lung surrounding the smaller air passages then undergoes a peculiar inflammatory reaction different from that of both lobar pneumonia and ordinary lobular pneumonia. There is much œdema of the interstitial tissue, often with capillary hæmorrhages, and both inter- and intra-alveolar exudate ; these changes bring about patchy consolidation and emphysema : the bronchial and mediastinal lymphatic glands are swollen and there is general mediastinal inflammation. The heliotrope cyanosis typical of fatal cases is an expression of the extreme anoxæmia which results from the general lung œdema. This anoxæmia may be responsible for the minor changes in other organs, or these changes, loss of tone of heart muscle, hyaline degeneration of voluntary muscle, cloudy swelling of liver and kidney, &c., may be due to toxic absorption from the lung. Later sequelæ, in cases not rapidly fatal, may be necrosis of the lung or a peculiar form of bronchiolitis obliterans.

The influenza bacilli are found in greatest purity and abundance in the innermost recesses of the respiratory tract, the alveoli and bronchioles. In the larger bronchi and, in the later stages of the disease, throughout the lung they may be accompanied by pneumococci, streptococci (*viridans* or *hæmolyticus*), staphylococci, meningococci, &c.

The tendency to capillary hæmorrhages and œdema in the inflamed lung is strongly reminiscent of Cecil and Blake's (1920) findings in monkeys (vide *ante* p. 347), and the conclusion is that human influenzal pneumonia is essentially similar to the experimental disease in these animals, and is, in many cases, if not in all, the expression of the pathogenic properties of the influenza bacillus.

Variations in Virulence.

There are many observations scattered through the literature, but no systematic study appears to have been made. The observations of Wollstein (1919) and McIntosh (1922) have already been mentioned.

In their experiments, as in most of those recorded, the quality investigated is the content of toxin in liquid cultures, and the wide variation which exists in this respect has been emphasized ; some strains are almost devoid of toxic action. Many observers (cf. Ishiwara, 1923) have noted the rapidity with which toxicity disappears in the course of artificial culture.

No comparative experiments have been made with the ' natural ' method of infection of Blake and Cecil (1920) which would place different strains in their order of infectivity as distinguished from toxicity. Such experiments would be most instructive from every point of view.

Immunization.

Experimental work on this subject also is very scanty. Parker (1919), Wollstein (1919), Ferry and Houghton (1919), McIntosh (1922), have all noted that a certain degree of immunity to the toxic action of influenza cultures can be produced by repeated injections into animals. Rabbits, for example, can be prepared so that they withstand 4 to 5 lethal doses of toxic filtrate. Passive immunization with the serum of such animals is less definite : one minimum lethal dose is about all that can be neutralized even by previous contact *in vitro*.

The production of antibacterial antibodies in experimental animals, already referred to under serological reactions (p. 342), though demonstrating that the influenza bacillus resembles other bacteria in this respect, is of doubtful application to possible immunity against infection of the respiratory mucosa. Here, again, further experimental work is urgently required.

The question of immunity against clinical influenza, acquired whether by an attack of the disease or as a result of vaccination with *B. influenzæ*, will be discussed in later sections (pp. 374, 375).

The Ætiology of Epidemic Influenza.

We have seen that influenzal pneumonia, a frequent complication of the pandemic disease, may rightly be regarded as an expression of the pathogenic properties of the influenza bacillus. But the nature of the causal agent of the epidemic itself is another matter, and this must now be discussed.

The 'Filter-Passer' Hypothesis.

The difficulties in making the known facts of the distribution of Pfeiffer's bacillus in health and disease fit the hypothesis that this bacillus is the ætiological agent of epidemic influenza and the failure to find any other probable bacterium have led many pathologists to predicate an ultramicroscopic filter-passing virus instead of a bacterial cause.

Experimental transference with bacteria-free filtrates to man. Kruse (1914) and Foster (1917) produced, by intranasal inoculation of filtrates of the nasal secretion of acute coryza, attacks of common cold in 15 out of 30 subjects (Kruse) and 7 out of 10 (Foster) ; no isolation of the subjects was attempted and the possibility of a mere increase of

susceptibility owing to the inoculation, or of purely accidental acquirement cannot be excluded. Similar experiments were made by Dold (1917), successful in the case of subjects living under ordinary conditions (Chinese students), but unsuccessful in the case of subjects kept in bed (hospital patients). Schmidt (1920) found that control inoculations with sterile saline produced more colds than inoculations with the filtrate. The results of Kruse and Foster in coryza, nevertheless, led to similar attempts with influenza. Selter (1918) produced slight influenza-like symptoms after one day of incubation in two subjects sprayed for half an hour with filtrate of throat washings from an influenza case ; no attempt to pass further the infection, if it was an infection, is recorded. Leschke (1919) sprayed three men with cultures from filtered sputum, throat washings and lung juice of influenza cases : all three took typical influenza 24 hours later ; the experiments were made at the height of the 1918 epidemic (September) : there was no isolation of the subjects. Nicolle and Lebailly (1919) injected filtrate of influenza sputum subcutaneously into two men, and intravenously into a third : the two developed mild influenza six days later ; the third did not.

Against these alleged positive results must be ranged those of Friedberger and Konitzer (1919) who entirely failed to produce illness in 26 persons sprayed with filtered influenza sputum and lung juice. Kruse (1918), the first defender of the filter-passer hypothesis, himself failed to produce influenza with filtered nose washings from a case of the disease. Lister and Taylor (1919) failed to convey influenza with filtered sputum (as well as with pure cultures of Pfeiffer's bacillus) though unfiltered sputum was successful (2 cases). Their experiments were performed with care to avoid a concurrent infection (isolation on an island), but some of the subjects may have been immune, as they had recently passed through an intense epidemic of influenza without falling victims.

Experiments on man, thus, give little support to the filter-passer hypothesis. Even if they had given many apparently successful results and hence deserved greater consideration, rigid proof would, in any case, be beset with almost insurmountable difficulties. For the experiments to carry conviction, one must provide a set of susceptible human subjects free from lurking infection, must maintain them in infection-proof surroundings during the course of the experiment, and, should the inoculation appear successful, must convey the disease to a similar set or sets of subjects with similar material from the first set. The history of experimental work on the virus of swine fever shows how great these difficulties are, even in the case of animals, and there is little hope that they will ever be overcome in the case of influenza in man.

Experiments on animals with influenza filtrates. These have also been quite inconclusive. Gibson, Bowman and Connor (1919) described lung lesions in monkeys after intranasal inoculation of filtered influenza sputum : these lesions, though inflammatory in character, were accompanied by very indefinite symptoms and may have had no connection with the

inoculation. In rabbits, they produced a hæmorrhagic condition of the lung, and were able to repeat the effect with filtered extracts of such lung : the known toxic effects of organ extracts on rabbits seem a sufficient explanation. Fejes (1919) had equally indefinite results with subcutaneous injection into monkeys of culture from filtered influenza sputum, while McIntosh (1922), with both filtered and unfiltered influenza material, both by subcutaneous injection and intranasal inoculation, had uniformly negative results with monkeys (15) and rabbits (8).

Culture experiments. In default of proof by experimental transmission, support of the virus hypothesis might be furnished by the demonstration of a living non-bacterial entity in culture media inoculated from the morbid material of epidemic influenza. Such an entity should be detectable in the cultures either by microscopical methods or by some physical or chemical change in the medium, and the appearances should be reproducible on inoculation into fresh medium. Many attempts in this field have been made.

Foster (1917) led the way with cultures of his filtrates from acute coryza. He used Noguchi's medium [unheated ascitic-fluid broth covered with liquid paraffin and containing a sterile fragment of fresh sterile kidney (rabbit or guinea-pig)]. Growth was recognizable in 24 hours by an opalescent cloud appearing in a layer just above the fragment of kidney : in this cloudy fluid minute coccoid bodies could be detected by prolonged staining with Giemsa's solution. (These cultures produced coryza in human beings in the same way as the original filtrates.) Dold (1917) made similar cultures and saw in them, by dark ground illumination, minute particles showing active movement. Von Angerer (1918) cultivated from the blood of rats, inoculated with influenza sputum, minute Gram-negative bodies which traversed filters and were regarded by him as filterable micro-organisms. Leschke (1919) saw similar bodies in cultures (in ascites broth) but failed to get them in subculture. Binder and Prell (1918) and Prell (1919) described the '*Ænigmoplasma influenzæ*', but Prell (1920) finally admitted that similar 'cultures' could be obtained from normal tissues. Gibson, Bowman and Connor (1919) observed in Noguchi's medium, inoculated with filtrate from influenza sputum, appearances similar to those described by Foster in his experiments on common cold. The coccoid bodies could be propagated indefinitely in similar medium, and could be recovered from the kidneys of inoculated rabbits.

These observations probably all depend on the same phenomenon ; it is well known that blood-cells, and, to some extent, the cells of other tissues, can set free in liquid surroundings minute particles possessing active Brownian motion and capable of taking on dyes. Olsen (1919) has shown that 'cultures' similar to all those mentioned above can be made regularly from normal blood, even after heating to 56° C., and it is obvious that the appearance of the 'coccoid bodies' in Noguchi's medium may not be the result of inoculation. Both Arkwright (1919) and Fildes

and McIntosh (1920) remark upon this in their destructive criticisms of the findings recorded above. Prausnitz (1920) suggests that some of the minute particles observed in filtrates may actually be derived from influenza bacilli which, when lysed, for example in the peritoneum of guinea-pigs, break up into a granular detritus. One may safely sum up by saying that none of the above attempts to demonstrate a living non-bacterial entity in filtrates of influenza material have succeeded.

There remain, however, among the culture experiments with filtrates of influenza material, those of Olitsky and Gates which are on quite a different plane. The reader is referred to the article by Dr. J. E. McCartney (p. 387) for a complete account of the *Bacterium pneumosintes* which these authors have described, and for an assessment of its relation to epidemic influenza. But it may be said here, in order to carry on the argument, that this micro-organism—though of great interest and importance to bacteriological science as one of a group of filterable bacteria of which few other members are known—has not yet been shown to occur sufficiently regularly in epidemic influenza, nor to produce sufficiently definite pathogenic effects, for it to have a serious claim to be the genuine and only ætiological agent.

Pfeiffer's Bacillus as the Cause of Influenza.

No proof has, thus, been forthcoming in favour of the virus of influenza being a filterable micro-organism and, though future discoveries may revive the hypothesis, at present it is one with no objective foundation. But, though Pfeiffer's bacillus starts with the initial advantage that it can be readily found and studied, there are definite and serious difficulties in accepting it as the primary agent.

Discussion of this question has in the past been conducted with unnecessary passion on both sides, and even now detractors and defenders of Pfeiffer's bacillus as the cause of epidemic influenza hold their opinions more by faith than reason. The truth is that a reasoned conviction is not yet possible ; it has to be admitted, when the pros and cons are carefully considered, that the absolute affirmative or negative will have to be decided by future work.

The question of the pathogenicity of *B. influenzæ* can be taken as settled ; hardly any pathologist now would affirm that it is invariably a harmless saprophyte. What is in dispute is the part it has played in epidemics and especially in the great pandemic of 1918–20, which caused the death of more human beings throughout the world than any plague hitherto known. Apart from its public health interest, epidemic influenza is rightly chosen as the critical point in estimating the importance of Pfeiffer's bacillus. Clinical influenza, a short fever with more or less inflammation of the respiratory passages, is, apart from epidemics, too vague a concept for the evaluation of bacteriological findings. It includes many cases of non-infectious or feebly infectious catarrhs which almost certainly have nothing to do with the epidemic disease, and there is no

lesion which the pathologist can say belongs to influenza only. Epidemicity is, therefore, the distinctive attribute of the pathological process to be investigated. But, even so, difficulties arise ; influenza-like epidemics vary both in quality and extent.

The periodic widespread prevalence of ordinary coryza which every town experiences from time to time is hard to distinguish from a mild form of influenza ; it is evidently epidemic though it is non-febrile and non-fatal. The influenza outbreak in the late spring of 1918 in Europe, though its morbidity was very high, was mild and attended by a low mortality ; the second and third waves in autumn 1918 and winter 1919, though their morbidity was less, were severe and fatal. And this difference was inherent, not seasonal, since New Zealand had the same date of maximum mortality as Great Britain. Was the first wave ' true influenza ' ? Were the others due to some more fatal virus of a different sort, to the same virus with increased pathogenicity, or to the same virus with concomitant spread of pathogenic bacteria ? In spite of these perplexing differences no one doubts that all the three waves of the pandemic can be called influenza, and it is especially with the bacteriology of these three that the discussion must be concerned.

Distribution of Pfeiffer's Bacillus in Health and Disease.

Some of the facts which have been used as a basis for the induction that Pfeiffer's bacillus is the cause of epidemic influenza have already been touched upon under various headings. Such facts are the bacteriology of the lesions, the experimental production of disease by pure cultures, the variations in virulence of the bacilli, and some of the immunity reactions. There remain to be examined (1) the data as to the distribution of the influenza bacillus in healthy persons, and in those suffering from influenza and other disorders during epidemic, pre-epidemic, and post-epidemic periods, and (2) the evidence as to serological types in its bearing on Pfeiffer's hypothesis. The value of these data is not merely that they serve as circumstantial evidence of the part played by the bacilli in epidemics. A more important consideration from the point of view of ætiology is that they fall into line with similar data in other epidemic diseases, e.g. cerebrospinal fever, diphtheria and scarlatina, in which no reasonable doubt exists that the respective bacteria are the agents primarily responsible. The interpretation of these data in relation to the rise and fall of epidemics is one of the principal tasks that lie before bacteriologists.

Distribution of B. influenzæ *in the pre-pandemic period (1896 to 1918).* The recorded findings refer to influenza bacilli in sporadic or endemic influenza, occurring in local and limited outbreaks and to their presence in other diseases, chiefly such as affect the respiratory tract. The latter class are of interest since records of the occurrence of Pfeiffer's bacillus in healthy persons during this period are scanty, and may be replaced to some extent by those in which non-influenzal disease was present. It is

not necessary here to give an extensive list of these findings. Kristensen (1922) gives an adequate summary and Donaldson (1922) tabulates them practically in full.

Wassermann (1900) reported that during the small epidemic in Berlin in the early months of 1900 he found the bacilli only in the first 24 hours of illness : he said that their disappearance coincided with the appearance of toxic symptoms, and supposed that the toxin was formed by the disintegration of the bacilli. On the other hand, Sacquepée (1901) in an epidemic in Rennes about the same date, in which 2,200 out of a garrison of 4,200 were attacked in two months, failed to find the bacillus until near the end of the outbreak, when it made its appearance, sometimes in great abundance. Scheller (1909) gives an account of the bacteriological findings in Königsberg during the height of an influenza epidemic in the winter of 1906–7, in which the lung lesions were identical with those described by Pfeiffer in the year 1892. Influenza bacilli were present in the sputum in 89 per cent. of 56 influenza cases, in 34 per cent. of 29 tuberculous sputa and in 23 per cent. of 109 swabs from the nasopharynx of normal people (20 of these had had influenza and gave 75 per cent. of positives). In the winter of 1907–8 influenza was less prevalent and the corresponding percentages were smaller ; in the summer of 1908 only 1 of 65 normal persons yielded influenza bacilli, and, in the winter following, the epidemic having quite subsided, 24 specimens of sputum from ' influenza ' cases were all negative (all pneumococcal), while in 90 tuberculous sputa and 95 examinations of the normal nasopharynx he failed entirely to find influenza bacilli. He says that the influenza outbreak spread later to the country districts, reaching its maximum in 1907–8, and that the frequency with which influenza bacilli were found in sputum from these districts increased correspondingly. Scheller was satisfied that these observations proved the ætiological relationship of Pfeiffer's bacillus to influenza, but it is obvious that the outbreak he studied had none of the typical rapid spread and short duration of epidemic influenza : it might rather be described as a local increase of respiratory catarrhs which even sceptics of Pfeiffer's theory of influenza admit may be caused by the influenza bacillus. Observations of this kind—the presence of Pfeiffer's bacillus apparently as the causal organism in inflammations of the respiratory tract—are fairly abundant throughout the pre-pandemic period. Eyre (1909) in London during the years 1902–9, found influenza bacilli *post mortem* in pure culture in 3, and along with other bacteria in 12 (total 15) out of 102 cases of ' primary ' broncho-pneumonia, while in the broncho-pneumonia following measles he found it in 2 out of 20 cases, in whooping cough in 2 out of 14 ; he failed to find it in 18 cases following diphtheria. Luetscher (1915) in Baltimore found Pfeiffer's bacillus in acute bronchitis in 22 out of 47 cases, in chronic bronchitis (6 of 13), broncho-pneumonia (22 of 42), lobar pneumonia (5 of 13), bronchiectasis (2 of 4), asthma (1 of 5), phthisis (3 of 21), laryngitis (7 of 38), coryza (6 of 38), accessory sinusitis (4 of 29), and in 7 of 60 excised tonsils.

He states that it was often obtained in pure culture and abundantly. Hübschmann (1915) found it *post mortem* in 31 out of 110 broncho-pneumonias in Leipzig during the first three months of 1915. Matthews (1915) found it commonly in respiratory catarrhs. The findings of Hammond, Rolland and Shore (1917), of Abrahams, Hallows, Eyre and French (1917) and of MacCallum (1919) have already been referred to (p. 350); they show that among both British and American soldiers the influenza bacillus was present and pathogenic in the year preceding the pandemic.

There were probably variations in the frequency with which influenza bacilli were present in different localities and at different times, even in the same locality in the pre-pandemic period. For example, Curschmann (1909) and Rose (1909) failed to find Pfeiffer's bacillus in an epidemic of influenza in Leipzig in the winter of 1907–8, whereas Hübschmann (vide *supra*) in the same town in 1915 found it abundantly.

In any case it is a legitimate statement that in the pre-pandemic period Pfeiffer's bacillus was not uncommon in the human air passages whether as a primary or secondary producer of disease. There are few observations on record as to its presence in the nasopharynx in healthy persons—Holt, quoted by Lord (1908) found it in 49 out of 254—but one may assume from its presence in a fair proportion of such diseases às tuberculosis and measles that it was to be found in health as well. There is evidence, finally, that the degree of its prevalence fluctuated in a given population from year to year, and could be correlated to some extent with the degree of prevalence of endemic catarrh. In the year immediately preceding the pandemic it was associated with an unusually severe type of bronchitis and broncho-pneumonia which appeared among soldiers, both in England and France, with such frequency as to resemble an epidemic.

As regards the more typically epidemic influenza, i.e. a comparatively mild, short fever, spreading widely with extreme rapidity and involving a large percentage of the affected population, there was a definite instance in the United States of America at the end of 1915. The epidemic extended from the Atlantic to the Pacific, attacking simultaneously so many people that business was carried on with much difficulty; Detroit, for example (population 500,000), had 100,000 cases of this influenza-like illness in the month of December, 1915. No great amount of bacteriological work appears to have been done in connection with this outbreak, but the health department of the City of New York stated that influenza bacilli were found in 9 out of 50 typical cases (=18 per cent.). The epidemic is of particular interest as a forerunner of the pandemic in 1918, which began, in Europe at least, as a similar mild disease of remarkably rapid spread. It may be an index that the acquisition of pandemic activity by the influenza virus, whatever its nature, is a gradual process the steps of which appear in the form of more limited outbreaks such as these.

Prevalence during the pandemic period. Some account of the pandemic itself is here necessary, the report issued by the Ministry of Health in

1920 being chiefly drawn upon. The period may be divided (for England) into three, the first wave in June, 1918, the second wave in October and November, 1918, and the third wave in March, 1919. Of these three, the first, as just mentioned, was composed for the most part of mild cases ; the type of disease was an acute, short fever with little or no respiratory catarrh, affecting simultaneously a large percentage of the population, perhaps as high as 10 per cent. and comparatively rarely resulting in severe pulmonary inflammation (less than 2 per cent.). In this wave, those of ages between 15 and 35 were attacked in higher proportion than those of other ages. The mortality, in the English civil population, was probably under 0·2 per cent. of all cases.

In the second wave, though there were many instances of the same mild type as in the first, perhaps as many as 80 per cent. of all attacks, a different manifestation of disease became prominent. This took two forms, (*a*) cases which started immediately with an acute pulmonary inflammation resulting in lung œdema, violet cyanosis, and death within a few days, and (*b*) cases which developed on the fourth or fifth day of an ' ordinary ' influenza a definite broncho-pneumonia, running the usual course of the ' primary ' broncho-pneumonia of pre-pandemic times and being followed, accordingly, either by death in the second week or by a long convalescence. The incidence on the population was almost as high as in the first wave, but there was less special incidence on the young adult. The mortality was about 3 per cent. of all cases (civilian England), i.e. 15 times that of the first wave, but was as high as 58 per cent. in certain groups such as ' institution ' cases in which complications followed.

The third wave had relatively few of the simple febrile type of case, but resembled rather an exaggerated prevalence of the endemic spring catarrhs known in pre-pandemic years with much bronchitis and broncho-pneumonia and a special incidence on those over 45. Taking all three waves together, probably 25 to 30 per cent. of the population of England had influenza, with a total mortality of 2·5 per cent. of those attacked.

Immunity to the disease in its second and third waves as a result of an attack in the first was by no means pronounced. The Leicester statistics, it is true, showed that those attacked in the first wave largely escaped the second ; but in Manchester, specially investigated on the ' block census ' principle, there was no evidence that the first wave had produced any immunity to attack in the second ; so much was this so that the hypothesis of a new virus, either developed locally or introduced, is tenable and must be considered in any discussion of the nature of the infecting agent in the pandemic.

Geographically the pandemic was world-wide, and it might be thought possible by careful attention to the date of the appearance in different countries, to trace the origin and the routes of transmission of a disease as highly infectious as influenza. It is in reality a matter of the greatest difficulty, and the most divergent views are held, each with some basis of probability.

The following facts appear to the writer to be significant. The earliest reports show that mild but highly infectious 'influenza' appeared in February, 1918, in the military camps in the United States, but failed to spread among the general population, which, as will be remembered, had gone through a similar epidemic two years before. In April a similar acute, but seldom fatal, disease broke out almost simultaneously in the civil population throughout France. In mid-April it appeared in the British Navy, and on the 10th of May, the point of maximum incidence, had affected 10 per cent. of the Grand Fleet. In the British Army in France the epidemic, after some local outbreaks at the bases (Rouen, &c.) in April, became definite towards the end of May and reached its maximum on the 25th of June. There were, probably, limited outbreaks of the same disease in the fighting line earlier, and Levinthal (1921) says he saw it among German soldiers on the Belgian front at the end of April. In all probability, not less than 10 per cent. of the combatants had mild attacks in this wave.

In Spain and Italy the first wave began at the end of April, apparently as an extension from France. The epidemic then spread to England, beginning almost precisely on the 29th of June, at which time it again became widespread, but still in the mild form, throughout civilian France, which, alone of all countries appears to have had two waves of this character. Holland, Switzerland, Denmark, Germany, Austria, Norway and Sweden each had a similar short but very extensive outbreak beginning a few days later than in England and reaching Sweden last (about July 10th). From Europe this mild but highly infectious disease spread throughout a large part of the Eastern hemisphere; in Egypt, Mesopotamia and India it began early (the end of June), in New Zealand and Australia towards the end of July, at which time it was also prevalent in China. In South Africa, and in all the countries of North and South America, no definite first wave was apparent; in these countries, though the first cases of the disease which appeared in September were not associated with a high mortality, the epidemic went on almost at once to become of the fatal second-wave type.

This second wave began almost simultaneously throughout the world in the beginning of October, 1918 (except in a few islands and in Australia, where a strict maritime quarantine seems to have delayed its appearance till January, 1919). Everywhere, in civilized as in uncivilized communities, in urban as in rural populations, the disease was widespread and attended by a high mortality. In India alone, 6 million deaths are reckoned as having occurred from influenza by the end of November, 1918, and, throughout the world, in the course of the whole pandemic, it is certain that there were more than 15 million fatalities directly due to it. The difference from the first wave and from the pandemic of 1889–90 is most striking; both these were distinguished by high morbidity, a large percentage of the population everywhere having an attack, but by an extremely low percentage mortality.

The third wave, though quite definite in England and most of the other European countries in the early spring months of 1919, had less of a world-wide character and may have depended more on seasonal conditions, the usual excess of respiratory catarrhs at this time of year being greatly exaggerated owing to the diffusion of latent infection as a consequence of the previous pandemic.

The bacteriology of the Pandemic Disease.

The epidemiological data are very confused and difficult to clarify; for example, it might quite well be maintained that influenza originated *de novo* in many different centres, perhaps as a gradual rise in virulence or in degree of distribution of the virus so that its pandemicity was not caused by world communications. But it cannot be said that the records of bacteriological findings are clearer or more consistent; this may be partly due to the difficulties of war-time bacteriology. In the first wave, in particular, the diversity of the published data is conspicuous and some reports are quite irreconcilable, e.g. those giving different results in the same town. Sobernheim and Novaković (1918) in Berne found Pfeiffer's bacillus to be practically absent from the early cases, in which pneumococci and streptococci appeared to be the prominent bacteria, whereas, in the second wave, *B. influenzæ* was found in pure culture in a large majority of the cases investigated (18 out of 23). H. Meunier (1919), on the other hand, during the first wave in south-west France in May, found Pfeiffer's bacillus in most of the mild cases, whereas after August, though it was still to be found at the onset of illness and in uncomplicated cases, the pneumococcus and streptococcus were the dominant bacteria in the fatal complications. In Hamburg, Olsen (1920[1]) found influenza bacilli in the lung *post mortem* uniformly in about 75 per cent. of cases (221 cases, of which 44 were in July, August and September), whereas Graetz (1919) and Schottmüller (1919) in the same town failed to find them in any but an insignificant minority, though apparently following an unexceptionable technique. Taking German work as a whole, it is evident that the majority of observers failed to find the bacillus with satisfying frequency in the first wave.

From British workers only a few bacteriological findings are on record for the first wave. Averill, Young and Griffiths (1918) made observations on 43 military cases in France between June 21st and July 10th, and found 32 specimens of sputum (out of 41 examined) containing Pfeiffer's bacillus. Martin (1918) observed 50 cases in the personnel of a base hospital in France in June. In 20 in which tracheal sputum was obtainable he found 8 showing influenza bacilli in plenty. Among the first 50 cases in the Air Force in London, Gotch and Whittingham (1918) saw influenza-like bacilli in the sputum in 62 per cent. but got Pfeiffer's bacillus in culture only in 8 per cent.; the predominating bacterium was *Micrococcus catarrhalis* (100 per cent.). Matthews (1918) in London, on the other hand, found *B. influenzæ* in all of 12 cases in July, using post-nasal swabs.

The Influenza Committee of the Advisory Board to the Director-General of Medical Services in France (1918) stated that ' at the beginning of the epidemic attempts . . . to isolate *B. influenzæ* from the sputa, nasopharynges and blood of cases were so seldom successful that doubt arose as to whether this organism was the ætiological factor in the disease '. But in the same report they bring forward observations by Shore and others on 76 autopsies (of which at least 30 were in June and July, i.e. towards the end of the first wave). Broncho-pneumonia of some degree was present in all except 6 lobar cases, and in 26 the predominant condition was ' purulent bronchitis ' (cf. Hammond, Rolland and Shore, 1917). Cultures were made from the broncho-pneumonic areas in 53 ; all yielded pneumococci and 40 *B. influenzæ*. The Committee thought that the difficulty in discovering Pfeiffer's bacillus in early cases might be attributed to the bacilli multiplying in the trachea and bronchi without producing an excess of reactive secretion, so that no sputum of deep origin appeared (but the sceptic might say that the absence of secretion prevents the epiphytic growth of influenza bacilli which occurs when secretion is in excess). They considered that association of the bacillus with influenzal pneumonia was striking and called attention to Martin's (1918) observations on the appearance of phagocytosis of influenza bacilli in the leucocytes of the sputum synchronous with the beginning of convalescence.

In Mesopotamia, Ledingham (personal communication) maintains that in the summer wave influenza bacilli could not be found in spite of satisfactory technique, whereas in India, Malone (1920) appears to have had no difficulty, though perhaps his observations were confined to the later severe cases.

In Spain, Falco and Tapia (1918) reported that among the early cases they found Pfeiffer's bacillus in 75 per cent. ; others were less successful, but de Salazar (1918) says that, speaking generally, it was present in the majority of cases.

One may summarize the bacteriology of the first wave by saying that, though the influenza bacillus was found in a fair proportion of the acute non-fatal cases typical of pandemic influenza, it is possible that this proportion coincided with the distribution of the bacillus in healthy persons in different localities at the moment of the outbreak. Its presence as a pathogen in the relatively small proportion of cases developing broncho-pneumonia might be conceded and regarded either as an autogenous secondary infection or as an example of concurrent transference of bacilli, fortuitously virulent, along with the unknown influenza virus.

In the second wave, a wave of high mortality with a conspicuous proportion of lung complications, the bacteriological findings are much more consistent. Practically all observers in all countries report an increasingly high percentage of cases in which Pfeiffer's bacillus was present in greater or less predominance over other bacteria. Only a few need be quoted. Among British writers on the army epidemic, Gibson and Bowman (1919) found influenza bacilli in 67 per cent. of specimens

of sputum and in the respiratory tract *post mortem* in 80 per cent., especially in the trachea and larger bronchi. The commonest accompanying bacterium was a *Streptococcus viridans* (88 per cent.) ; pneumococci were practically absent. Tytler, Janes and Dobbin (1919) found the influenza bacillus almost constantly in their post-mortem material (60 out of 66 cases) ; in 45 per cent. it was the predominant organism and in many cases it was almost the only one ; hæmolytic streptococci were rare (3 cases), but pneumococci were present in at least 25 per cent. In the 52 cases showing purulent bronchitis Pfeiffer's bacillus was found in 92 per cent., and in the peri-bronchial abscesses in 97 per cent. Patterson, Little and Williams (1919) studied 46 consecutive fatal cases in the late autumn of 1918. *B. influenzæ* was recovered from the bronchi or lungs from every case but one ; infection with pneumococci and streptococci, in addition, was commoner in the cases of longer duration. In the Navy, Fildes, Baker and Thompson (1918), who failed to find influenza bacilli in July and August—but this, they think, may have been due to unsatisfactory medium (human blood-agar)—now found them in the sputum of 12 of 15 uncomplicated cases and in practically all their post-mortem material. Among civilian patients, McIntosh (1918), who failed similarly in the early summer, now found them in 8 out of 12 examinations of the nasopharynx in uncomplicated cases and in the sputum of broncho-pneumonia in 21 out of 25 ; pneumococci were notably absent. In a later series of post-mortem examinations, McIntosh (1922) found influenza bacilli in 94 per cent. Muir and Wilson (1919) among soldiers, both American and British, found them *post mortem* in all of 22 cases of broncho-pneumonia, and remarked that the bacilli were in special relation to the lesions (of which they give an excellent description).

In South Africa, Lister and Taylor (1919) found influenza bacilli in 53 out of 56 fatal cases of broncho-pneumonia. In India, Greig and Maitra (1919) found them especially in the purulent exudate of the nasal sinuses and constantly along with pneumococci in fatal cases. Liston, quoted by White (1919), also found this association with pneumococci in Bombay.

In the United States of America, Williams, Hatfield, Mann and Hussey (1919) made observations on the epidemic which began in New York in September, 1918. Swabs from the nasopharynx in the early acute stage showed influenza bacilli in almost all of 200 cases ; the earlier the examination the more abundant were the bacilli. In 30 marines attacked simultaneously by influenza Pfeiffer's bacillus was found in all without exception, while in 30 autopsies, 24 yielded influenza bacilli in the lung, 5 in pure culture ; in 26 out of 27 scrapings from the trachea, the bacilli were present in large numbers. Pritchett and Stillman (1919), during the last four months of 1918 in New York, found influenza bacilli in 83 per cent. of uncomplicated cases of influenza and in 93 per cent. of those with broncho-pneumonia. They noted that their frequency in the

respiratory tract rose and fell with the intensity of the epidemic. Dick and Murray (1919) in Chicago made somewhat similar observations, their positive findings in influenza patients diminishing from October, 1918, to January, 1919, in the order of 74, 54, 43 and 36 per cent. Duval and Harris (1919) in New Orleans found influenza bacilli in all of 75 patients, and in the lungs in 16 out of 17 fatal cases. McClelland (1919) in the first 10 days of the epidemic in Louisiana (25th September to 3rd October), found the bacilli in the nasopharynx in 1,749 out of 1,919 patients (91 per cent.). In Denmark, Kristensen (1922) found the bacilli in about 30 per cent. of sputa from influenza patients ; in the summer wave he had found them in less than 10 per cent.

In Germany, Leichtentritt (1918) found Pfeiffer's bacillus during the autumn epidemic in Breslau in 24 out of 36 cases of influenzal pneumonia examined *post mortem*. Michaelis (1918) in Berlin failed to find it in the first week of the summer epidemic but found it with increasing frequency later. Messerschmidt, Hundeshagen and Scheer (1919) found it in 18 of 20 samples of sputum in September, whereas in June to August they found it in only 47 of 86 ; in the later months the typical microscopic picture of masses of the bacilli in sputum became common while formerly rare. Fraenkel (1918) in Heidelberg failed to find the influenza bacillus in the summer epidemic, but in the autumn found it in 8 of 11 autopsies.

French workers have recorded few observations on the bacteriology of the lungs during this portion of the pandemic. Aïtoff (1918) found pneumococci in symbiosis with Pfeiffer's bacillus, the latter being present in the great majority of cases, during the epidemic in Archangel which followed the arrival of American troops in September, 1918. In Rome, Carpano (1919) came to the conclusion that the influenza bacillus was invariably present at the beginning of the disease but difficult to find later when secondary infections had occurred. He studied only 12 cases, but his article is full of interesting observations on the morphology and culture of Pfeiffer's bacillus.

These high percentages of demonstrable infection with Pfeiffer's bacillus are impressive, and appear to justify the inference that, whatever the nature of the true influenza virus, this infection with *B. influenzæ* was the direct cause of the enormous and world-wide mortality of the late autumn of 1918. No other bacterium was found with such constancy, though, in different units of population, the pneumococcus, *Streptococcus hæmolyticus*, *S. viridans*, &c., might predominate or be comparatively rare.

Influenza bacilli in normal persons during the pandemic. I have found no observations recorded as to this point in the first or summer wave. During the autumn wave observations are fairly numerous. Siler (quoted by Cummins, 1919, found that 27 per cent. of 2,179 healthy persons in medical units of the American Army in France were carriers of influenza bacilli. Dudley (1919), on a hospital ship in which there were continually many cases of influenza from October, 1918, to April, 1919, found that 90 per cent. of the nursing staff (29 men) were carriers on one or more

examinations, while the crew had 28 per cent. (32 examined). In November, 1918, Fildes, Baker and Thompson (1918) found 15 carriers (21 per cent.) among 71 apparently healthy men ; later Fildes (1920) found 18 carriers out of 76 boy entrants to a training establishment, and in the spring of 1919 64 per cent. of 177 healthy men in barracks. Scott (1922), in November, 1918, found 40 per cent. of 139 out-patients in London hospitals were harbouring influenza bacilli in their nasopharynx. In Germany, Neufeld and Papamarku (1918–19) found only 2 carriers out of 25 healthy people during the epidemic. Messerschmidt, Hundeshagen and Scheer (1919) failed to find a single carrier in 100 healthy soldiers in barracks where influenza later appeared. Löwenhardt (1920), on the other hand, gives 25 per cent. as the proportion of carriers of influenza bacilli in 289 non-influenzal tuberculous patients during June to December, 1918. The percentage dropped in succeeding half-yearly periods to 16, 7 and 5 per cent. In America, Williams *et al.* (1919), during the height of the epidemic in October, 1918, found only 2 carriers among 34 healthy girls from a home in which no influenza cases had occurred ; the two carriers both had slight colds. Pritchett and Stillman (1919) examined the throats of 177 normal persons (personnel of the Rockefeller Institute), and found 74 carriers (42 per cent.). Winchell and Stillman (1919) found that this percentage persisted during the succeeding six months—December, 1918, to June, 1919. They found that healthy carriers harboured influenza bacilli for as long as five months. Kristensen (1922), in September, 1918, found 23 per cent. of carriers among 142 recruits: similar examinations during succeeding years to 1922 gave nearly the same proportion. He observes that the percentage of healthy carriers and the prevalence of influenza in Denmark ran parallel during these years.

The Post-Pandemic Period.

During the post-pandemic period, 1920 onwards, smaller epidemics have been described in several countries ; Edington (1920) in England, Kristensen (1922) in Denmark, Preuss (1921) in Germany, Small and Stangl (1920) in America have given such descriptions. Similar high percentages of findings of Pfeiffer's bacillus were the rule. In addition, sporadic cases of influenzal pneumonia with the characteristic morbid anatomy and presence of the influenza bacillus have been frequently seen by pathologists in this country during routine autopsies (personal communications). A definite correlation between the percentage of influenza cultures from pathological respiratory sources and the prevalence of epidemic influenza as measured by the number of deaths recorded from it, has been established by Glynn and Digby (1923) for the City of Liverpool in 1921. Similar observations were made by Patterson and Williams (1922) in Melbourne.

As regards healthy carriers, Edington (1920), in the presence of the epidemic he described, found 18 per cent. of these, whereas when it was over, he found only 6 per cent. Preuss (1921) found 10 per cent. in the

summer of 1920, and only 3 per cent. in the succeeding winter. My own observations (partly unpublished) show percentages in the normal nasopharynx in the winter months varying from 70 per cent. in 1920 to 50 per cent. in 1925. Topley (personal communication) found in monthly examinations of a group of about 100 persons in Manchester that the percentages of findings of influenza bacilli were 37 per cent. in July, 1925, and 70 per cent. in September, 1927 ; the fluctuations between these dates are to some extent correlated with season, the higher figures being in winter and spring, the lowest figure, 31 per cent., occurred in October, 1926, and the highest, 80 per cent., in March, 1927.

Interpretation.

These, then, are the facts recorded as to the distribution of the influenza bacillus in health and disease and in epidemic and non-epidemic periods. What is their significance ? Two main interpretations are possible. On the one hand, it may be held—and Kristensen (1922) has stated the case with great force—that the distribution of the bacilli in the epidemic disease is merely the consequence of a similar epiphytic distribution in the pre-epidemic period. His theory may be expressed as follows.

Theory of Pfeiffer's bacillus as an epiphyte and secondary invader. It is supposed that the bacillus finds the most favourable conditions for its development in catarrhal mucous membranes. Such catarrh may be produced by many different infective agents, and perhaps, by non-infective physical irritants, e.g. ' Influenza ' in its widest sense, whooping cough, measles and ' simple ' bronchitis. In normal mucous membranes conditions are less favourable for its continued existence, but, as there is a continuous supply of the abnormal owing to the prevalence of the diseases cited, there will always be centres of diffusion of the bacillus to keep up a certain low level of infestation with it among the normal population. In the first wave of pandemic influenza only this small percentage among the patients will yield influenza bacilli, but, owing to the great increase in the proportion of the population whose influenza attack has made their mucous membranes a suitable nidus, the percentage of carriers of influenza bacilli will rapidly rise. Coincident with this rapid rise and the consequent greater frequency of transference of the bacillus from person to person, it acquires greater growth energy and may not only multiply as an epiphyte in normal throats —which it rarely does in non-epidemic times—but may gain the power itself to produce either catarrh or a disease resembling influenza, or, especially, the pneumonic sequelæ. As the pandemic dies down the conditions of its distribution slowly return to normal. This ' normal ' may vary in different places so that, when 'ripples of influenza ' occur in interpandemic periods, all grades of bacteriological findings may appear, from complete absence of Pfeiffer's bacillus in the influenza patient to almost constant occurrence.

It will be observed that none of the known facts are discordant with this hypothesis, and, in particular, that it provides for an acquired

pathogenicity of the influenza bacillus associated with increased frequency of its occurrence in the lung, and responsible for the increased fatality of the disease later in the pandemic. It will be noted, also, that the theory limits the pathogenic activity of Pfeiffer's organism to these later stages and to a secondary role in the causation of the epidemic disease. The hypothesis furnishes, also, the simplest explanation of the diversity of serological types found in patients suffering from influenza ; the different types are simply descendants of similarly diverse types which were present as epiphytes in the population before the influenzal outbreak.

Should the hypothesis be correct, one would have to postulate for influenza itself, as an infectious disorder, an unknown virus capable of assuming suddenly the power of rapid dissemination. The cause of this increased infectivity may be supposed to be a casual rise of virulence in a hypothetical focus ; a general secular fall of resistance in the rest of the world towards the end of the interpandemic period may also be a necessary condition.

This theory has certainly a strong claim, and it would be very rash to say that it is inadmissible. But it must be pointed out, in the first place, that it needs some assumptions which may not be justified and, secondly, that an alternative hypothesis is available.

As regards the doubtful assumptions, the least vulnerable is that which postulates that the diffusion of the influenza bacillus among influenza patients at the beginning of the epidemic is really of small extent (corresponding to the diffusion among the population just before). The objection that technical errors were responsible for the almost universal failure to find the bacillus in a decisive proportion of cases in the summer outbreak of 1918 seems improbable ; one must accept as a fact the absence of influenza bacilli (in a condition fit for growth on artificial media) in some, at least, of the early cases.

Another assumption is that, in their natural habitat, the respiratory mucus, influenza bacilli are stable in antigenic composition ; should this not be true, the argument that the diversity of the serological types in the disease proves their origin from similar pre-epidemic epiphytic strains loses its validity, and with it falls the argument that the non-existence of a single type or a restricted number of types predominant in the epidemic shows that the influenza bacillus is not the true epidemic agent.

Again, the postulate of a primary focus lacks corroborative evidence. Pre-pandemic diffusion of the causal agent with multiple foci of raised infectivity would appear to be at least equally capable of explaining the epidemiological facts, so far as they are known. For Pfeiffer's bacillus such pre-pandemic diffusion is established ; for the unknown virus there are naturally no data.

Finally, the assumption of an invisible virus has to contend with the incompatibility of such facts as are known about the properties of zymotic viruses, especially their high specificity (e.g. small-pox) and the corresponding immunity following upon an infection. In epidemic influenza,

though some degree of such immunity is indubitable, there is a very large residue of failure of immunity as shown by repeated attacks in successive outbreaks. This failure is compatible with a bacterial cause such as Pfeiffer's bacillus, but not, on present knowledge, with a ' virus ' (except, perhaps, that of foot-and-mouth disease).

Theory of Pfeiffer's bacillus as the primary cause. The alternative theory is that the *B. influenzæ* (Pfeiffer) is the primary cause of pandemic influenza in all its manifestations ; the differences in severity of separate phases of a pandemic would be ascribed to differences in toxicity, in invasive power and in resistance to the self-protective activity of the human host on the part of different strains of the bacillus.

Some evidence of the existence of such differences has been obtained by experimental work (vide p. 346) ; the full analysis of these differences awaits investigation, but the analogy with other pathogenic bacterial species leaves little doubt of their possibility. The characteristics of the first wave of the 1918 pandemic—and of the uncomplicated disease in general—would be explained as the result of infection with an influenza bacillus possessing enhanced power of multiplication in the normal respiratory tract and a high toxicity, but poorly endowed with defensive qualities against the bactericidal properties of the inflamed mucosa ; such a set of attributes would account for the high morbidity rate, the acute symptoms, the rapid recovery and the absence of the majority, or all, of the bacilli when bacteriological examination is made. This last point, the possibility of an infection with Pfeiffer's bacillus producing disease, but being followed by rapid and complete disappearance of the bacilli, unfortunately, but almost necessarily, lacks the support of objective evidence. Wassermann (1900) and Williams *et al.* (1919) certainly suggest that such a phenomenon occurs, and there are many observations of sputum showing abundant influenza bacilli on microscopical examination but yielding none in culture. There can be no doubt that in some specimens of sputum the bacillus will live for days outside the body, whereas in others it dies within an hour or two of expectoration.

The peculiar fatality of the later stages of the pandemic would mean that some strains of influenza bacillus then being diffused throughout the population had retained their infective and toxic qualities, but had acquired a greater resistance to the bactericidal power of the respiratory secretions ; accordingly, they would continue to multiply on the mucous membrane and produce a more intense and lasting toxic action. In addition, of course, the diffusion of secondary invaders, especially the hæmolytic streptococcus, would be responsible for excessive mortality in at least some sectors (vide *infra*).

How does the diversity of serological type in the bacilli found in influenza affect the theory of their causal connection ? There is no doubt that, if it did not exist, if a single type or only a few were found in the epidemic disease as compared with sporadic cases and healthy carriers, the fact would be used as a strong argument in favour of the influenza

bacillus as the causal bacterium. On the other hand, it is established that, in other epidemic diseases such as diphtheria, scarlatina and cerebrospinal fever, a very considerable diversity exists in the serological types of the causal organisms. The cerebrospinal fever epidemic of 1915–16 in Britain was particularly studied from this point of view (Eastwood, Griffith and Scott, 1916, 1917). That epidemic should be looked upon as primarily a meningococcus infection of the upper respiratory passages ; presumably new races of meningococci, whether autochthonous or imported, had come into existence, possessing high infectivity and power to colonize the respiratory mucous membrane, with or without resulting catarrh. In this sense the epidemic affected a large proportion of the population ; in a very small minority the invasive power of the infecting strain was so great, the resistance of the infected person so small in relation to it, that meningitis resulted (and probably a general blood infection as shown by the petechial rash). These cases, the clinical cerebrospinal fever, should be regarded as incidental to the epidemic meningococcus throat infection, just as sparks may be thrown up from a widespread slow combustion. Now the meningococci responsible for the epidemic so defined were serologically highly diverse ; the number of separable types to be found in the nasopharynx of the people as a whole, and, to a less extent, in cases of the disease, was limited only by the amount of labour required for their identification. It is true that certain types were much commoner in the cases of actual meningitis, especially among soldiers, than among carriers in the general population, but, when all strains from meningococcal meningitis were considered, the full range of serological variation among them was almost as great as among the strains from the nasopharynx.

No large epidemic of diphtheria has been studied in the same way, but a diversity of similar degree exists among the strains from cases due to its endemic prevalence (Eagleton and Baxter, 1923 ; Smith, 1923 ; Scott, 1923). The same applies to scarlatina, in which many serological types of toxic streptococci have been identified (Smith, 1927 ; Griffith, 1927).

Whether this diversity is a defensive response of the infecting bacterial species, an attempt to avoid bacterial antibodies developed by the human host, as I have suggested (Scott, 1923) for the diphtheria bacillus, is a speculation which does not require discussion here. The fact remains that, in these diseases—all of which are of epidemic nature but are built upon a foundation of endemic distribution of the causal organism —diversity of serological type is the rule. In the case of meningococcus epidemics, the diversity is less apparent at first sight owing to the selective process constituted by the meningeal infection, which segregates (for the bacteriologist) the strains of highest virulence, the so-called ' epidemic types ' ; in other diseases, e.g. diphtheria, in which special virulence is not associated with special anatomical localization of the infection, ' epidemic types ' are difficult or impossible to establish. The conclusion is that the serological diversity of the influenza bacillus is in conformity

with a similar diversity among the bacteria responsible for other epidemic diseases in which the primary site of infection is the upper respiratory tract. It certainly cannot be used as an argument against Pfeiffer's bacillus as the prime cause of epidemic influenza.

How is its inter-pandemic diffusion to be reconciled with the hypothesis that Pfeiffer's bacillus is the pandemic agent? The following argument shows that, not only can it be explained, but that such diffusion is a necessary condition for its causal relation to epidemics. Since there is no evidence of a permanent geographical focus in which influenza is endemic and from which it overflows to create the pandemics, and since there is no evidence that the infecting agent can exist outside the human body, one must assume that, whatever be its nature, whether 'virus' or influenza bacillus, a certain low-grade diffusion must exist in many communities throughout the inter-pandemic period. It follows that, whatever the infecting agent, it must be subject to rise and fall of pathogenic power and that it can exist in the feebly pathogenic phase, without dying out altogether, in, at least, some sections of a community. One cannot say whether these conditions are fulfilled by the unknown virus, but it is evident that the facts known about the diffusion of Pfeiffer's bacillus do so fulfil them. As was indicated in discussing the virulence of the bacillus, further investigations on fluctuation in this respect are much needed, and an enquiry into the class of population in which its continued existence is best maintained would also be of great interest. There is evidence that the zymotics associated with respiratory catarrh, measles and whooping cough, furnish such an element; cases of pulmonary phthisis also appear to provide a lasting reservoir of the bacillus. Apart from these diseases there is some evidence that children generally and adults of advanced age are more commonly 'carriers' than those in the prime of life.

Objections to the Pfeiffer theory. I have attempted in the preceding section to reduce the difficulties in accepting the Pfeiffer theory. In particular, the pre-pandemic diffusion of the bacillus and its presence during a pandemic in people who have escaped the disease, the serological diversity of the bacilli isolated from cases of epidemic influenza, and the different character of successive waves have been shown to be difficulties capable of theoretical explanation. The difficulty that in some of the sporadic cases of clinical influenza and even in some of the inter-pandemic outbreaks, the influenza bacillus may fail to be detected, or be, in fact, absent, may be accounted for by supposing that other bacteria, the pneumococcus, the meningococcus group (including *M. catarrhalis*), the streptococci, &c., may on occasion produce a syndrome clinically like the pandemic disease, and may even possess epidemic striking power. But the prime difficulty, the absence of the influenza bacillus in some, at least, of the pandemic cases, has not received an explanation which compels acceptance. The suggestion that in these the bacillus infects, multiplies and, in the act of its own dissolution, provides the poison which produces disease, remains a surmise with but little objective basis and no sure

analogy in the pathology of other diseases of bacterial origin. It may be the true explanation, and be some day proved, for example, by intensive bacteriological investigation of susceptible persons during epidemics, so as to catch the disease in its incubation and explosion, but, until that has been done, the case for Pfeiffer's bacillus as the essential ætiological agent of influenza is not complete, however probable it may seem on other grounds.

Lines of Future Work.

It will be apparent from the foregoing discussion that, in the writer's opinion, the solution of the problem of the immediate cause of influenza as an epidemic disease lies in further study of *B. influenzæ*, the range of its variability in virulence and resistance to the human defensive mechanism, its distribution in communities which, except in pandemics, are free from influenza-like disease and particularly its behaviour on introduction in a virulent form into the respiratory passages, whether experimentally or in ' natural ' infections.

Research in other directions is equally desirable; notably one might seek some ' third factor ', conceived as the qualifying condition for an acquired pathogenicity among bacterial epiphytes. Or the problem might be attacked indirectly by further investigation of swine-fever, in which *B. suipestifer* might be considered as playing a part analogous to that of the *B. influenzæ* in influenza, and an attempt made to establish an invisible phase in the life-history of the bacteria which would represent the invisible virus.

The ætiology proper of epidemic influenza, i.e. the reasons for its epochal appearance, cannot be discussed here. It is obvious that proof of the direct cause of the disease would greatly assist investigation of this question, at present so obscure, but would not necessarily bring its solution. The subject has been admirably discussed by Greenwood and Carnwath (Report on Influenza, 1920), and the reader is referred to them for a full consideration of the problems and the lines on which may be sought their explanation.

Mixed Infections in Influenza.

The part played by the bacteria which so often accompany the influenza bacillus in natural infections is not yet capable of precise evaluation. Several of them are themselves potentially pathogenic, though common enough in the normal mouth, nose and throat. The association is irregular; sometimes it is a pneumococcus, sometimes a streptococcus, sometimes a meningococcus which accompanies Pfeiffer's bacillus, and this irregularity suggests (1) that the influenza bacillus is the leading partner and prepares the way for the others, and (2) that their presence may be a matter of chance, depending on their pre-existence in the person infected by the virulent influenza bacillus or in the person directly conveying infection to him. In some cases the association is firmer and, in a given outbreak, influenza bacilli and hæmolytic streptococci, for

example, may be the regular finding. In such instances one may assume that the accompanying bacterium is also of heightened virulence, itself takes part in the production of disease and gives a special character to the clinical symptoms and pathological changes. It is on observations of this kind that the theory of the ' complex virus ' as the cause of influenza epidemics has been based. This theory in its various modifications has been regarded with favour by several medical philosophers, who see in it the immediate and simple explanation of the divergent bacteriological findings. Some would require an invisible virus or even a ' miasma ' as the primary impulse to acquired pathogenicity of the bacterial epiphytes, which then act as a mixed infection and are immediately responsible for the production of disease. Others think that simple symbiosis of bacteria of low pathogenicity may, under unknown conditions, lead to enhanced and stable virulence of the mixture alone or of one or more of its members. It is difficult to reconcile such theories with the remarkable uniformity of influenza as a world-wide simultaneous pathological process.

Experimental observations, however (cf. p. 348), do support the conclusion that infection with a mixture of influenza bacilli and other bacteria, especially streptococci, may confer an increased infecting power on both the members of the mixture or on the influenza bacillus alone—the former effect in the case of streptococcal, the latter in the case of pneumococcal mixture (Hudson, 1924). It may well be admitted that in the production of the complications of influenza this symbiotic effect plays a definite part. Whether it has anything to do with the nature of the primary influenza virus is a question which can only be settled when a final decision is reached as to the causal role of Pfeiffer's bacillus itself.

Conditions determining Transference of Influenza Bacilli.

Outside the human body the influenza bacillus is exposed to the deleterious influences of drying, direct or diffuse sunlight and absence of suitable nutrient material ; the length of its survival suspended in the air or attached to particles of dust is probably much less than 24 hours. Transport of the infection over long distances by air currents must, therefore, be of quite minor importance. In sputum at atmospheric temperature and protected from drying and light, survival may be much longer, probably as much as 10 days (Ricciardi, 1904, quoted by Scheller, 1913), so that transport on infected articles of clothing (handkerchiefs especially), food utensils, &c., is a possibility which cannot be excluded, though infection from such articles is probably not easy. There is no evidence that domestic animals provide suitable conditions for the survival of the bacillus ; for example, heavily infected mice (inhalation experiments) no longer harbour influenza bacilli after 24 hours.

The chief mode of transport is, no doubt, by minute droplets of saliva and respiratory mucus discharged in speaking, coughing and sneezing. The transporting power of these vehicles for the bacillus is limited both in space and time (vide discussion of droplet infection in

Vol. VI) so that, for practical purposes, it can fairly be said that the transference of influenza bacilli is direct from man to man and not by any intermediary agent.

Within the human body, especially in the air passages, the influenza bacillus may, in certain circumstances flourish indefinitely. In pulmonary phthisis, for example, the sputum may contain Pfeiffer's bacillus in great numbers for years; in other chronic inflammations, sinusitis, chronic bronchitis and bronchiectasis, similar findings have been recorded; in some of these latter conditions the bacillus itself may be the cause of the chronic inflammation, the local pathological process being in the nature of a balance between the vitality of the bacterium and the reaction to it of the host. In normal persons a similar prolonged duration of the carrier state is probably much less common; generally speaking, the healthy mucous membrane provides relatively unfavourable conditions for the multiplication of the bacillus, as is shown by the scanty colonies which appear in cultures from such a site. But the persistent high carrier rate demonstrable in one group of people (vide p. 364) is practically convincing proof that, under certain circumstances, the bacillus may survive for months in apparently healthy persons; there are also records of prolonged carriage after artificial inoculation with Pfeiffer's bacillus which support this view (vide p. 349).

The circumstances determining this persistence in apparent health must be complex; they represent the resultant of two activities, the resistance to its environment of the particular strain of the bacillus—a quality not necessarily correlated with the fluctuating attributes known as virulence and infectivity—and the defensive mechanism of the tissues and their secretions, which limit the extent of invasion. In all probability this balance does not differ in essence from that in the chronic inflammations, but has been struck at a higher level of advantage for the host. (There is some evidence that the upsetting of such a balance in favour of the bacillus by fatigue, chill or unhealthy air may be the true pathology of many cases of coryza, which, bacteriologically, would then be autogenous and not the result of infection from without. For a full discussion of the carrier state in general and its consequences the reader should consult the section devoted to this subject.)

Fluctuations in the carrier rate among normal people undoubtedly occur (cf. p. 364), and are of great epidemiological interest. Whether they are to be correlated with fluctuations in the resisting quality of current strains of the influenza bacillus or with a rise and fall in the efficiency of the defensive mechanism in the human population concerned or whether the two processes are interdependent are questions which cannot yet be answered.

Overcrowding must be a potent factor in raising the carrier rate in the population subjected to it. Its effect in the case of the meningococcus has been studied by Glover (1920), who has shown that dormitory conditions are of chief importance in this respect, and that proper spacing of beds

can lower the carrier rate. He has shown also that by thus diminishing the amount of cross-infection, the incidence of cerebrospinal fever can be lowered or abolished. The indication is that frequent nasopharyngeal cross-infection is the process by which rise of virulence is achieved by the meningococcus. No similar study has been made in the case of the influenza bacillus, but there can be little doubt that the data obtained for the meningococcus apply also to it.

Immunity to Infection with Influenza Bacilli.

As things are, with Pfeiffer's bacillus still on trial as the cause of influenza, the facts known about immunity to the disease cannot be translated in terms of immunity to the influenza bacillus. The agglutination reactions of the serum of convalescent influenza patients, described on p. 341, are not inconsistent with the Pfeiffer theory, though they may be interpreted as the reaction to the bacillus in its capacity as a mere secondary invader. Levinthal (1921) makes the point that the only classes of the population which showed some degree of immunity to the 1918 epidemic were (1) children, (2) adults in the later decennia, (3) those who had recently had measles and (4) those suffering from pulmonary phthisis ; all these are classes which display a high percentage of infection with influenza bacilli in non-epidemic periods and may thus be supposed to have developed and maintained at a high level an immunity to fresh infection with the epidemic strains. The relative or absolute immunity of ' carriers ' is a subject of great interest in connection with all epidemic diseases, but cannot be discussed to advantage here (vide Vol. VI). In future epidemics the technical advances which have been made in the matter of detection of the influenza bacillus may settle this question by showing that carriers found in the early stages of the outbreak escape entirely or furnish the milder cases.

The question of immunity to influenza in the 1918 epidemic has been fully discussed by Greenwood and Carnwath in the Report on Influenza of the Ministry of Health (1920), q.v. They show that there is evidence of immunity to attack in the second and third waves acquired by an attack in the first, and some suggestion that there were type distinctions in the immunizing value of outbreaks at different times and places. These phenomena are, of course, compatible with the Pfeiffer theory, but the bacteriological data are quite insufficient for critical examination in terms of it.

Practical Diagnosis.

It is obvious from all that has been said that, at present, the diagnosis of influenza by bacteriological methods has no sure foundation. Neither the apparent absence nor the finding of influenza bacilli in the respiratory passages gives an unequivocal answer. It is possible that a continuous record of the carrier-rate for Pfeiffer's bacillus over long periods of time in a given population may furnish significant facts and, especially, may give warning of the approach of an epidemic due to this bacillus, but,

until the varying pathogenic quality of different strains can be evaluated, the prospects of success with such a procedure are poor.

As regards identification of the bacillus, especially when found in unusual situations, the tests of dependence on growth factors and the precautions necessary in their application have been described on pp. 333–9. Differential diagnosis is particularly required against *B. pertussis*, *B. ducrey*, and certain Pasteurellas which may on first isolation simulate dependence on blood in the nutritive medium.

Hygienic Measures.

Prophylactic inoculation. A review of the question of vaccines in the prophylaxis of influenza is given by Jordan (1927). He concludes that none of the evidence in favour of vaccine prophylaxis in influenza is satisfactory, while carefully controlled observations on the effect of *B. influenzæ* alone as a vaccine have given absolutely negative results. Mixed vaccines have been chiefly used, however, with the intention of raising immunity to pneumococci, streptococci and other bacteria which act as secondary invaders. This procedure is theoretically unassailable, except that the composition of such a vaccine must at present be purely empirical. There are no solid data as to the practical value of the many varieties of mixed vaccine which have been used in the hope of conferring such protection.

General hygiene. Measures based on the fundamental assumption that the disease is conveyed by secretions from the mouth and nose are (1) prevention of overcrowding, (2) isolation of the sick, (3) the wearing of masks during epidemic periods. In practice neither (1) nor (3) can be effectively carried out in communities organized as they are to-day, while (2) would in many cases be applied too late, the period of greatest infectivity being probably the first few hours of illness. Practical hygienic measures to combat those diseases which enter by the respiratory tract are extremely difficult to conceive and apply ; they form one of the greatest problems for future preventive medicine. In the extension of the sense of social impropriety to such acts as unguarded coughing, sneezing and loud talking lies, perhaps, the chief hope of a reduction of massive droplet infection and consequent respiratory disease.

B. koch-weeks.

Historical.

While engaged on cholera investigation in Egypt in 1883, Koch described one variety of the prevalent Egyptian conjunctivitis in which microscopical examination of the pus showed great numbers of minute rods; culture was not attempted. In 1887, Weeks, in New York, described similar bacilli in acute contagious conjunctivitis ; these grew on ordinary half-per-cent. agar, but only in association with a diphtheroid bacillus. Mixed cultures of this kind reproduced the disease when inoculated into the human conjunctiva, whereas pure cultures of the diphtheroid did not.

The minute bacilli are now known as *B. koch-weeks* (*Bacterium ægyptiacum* Lehmann and Neumann). Later workers, especially Morax (1899) and Weichselbaum and Müller (1899), produced the typical conjunctivitis with pure cultures. The bacillus was, at first, regarded as peculiar to the conjunctiva and distinguishable from the *B. influenzæ* (Pfeiffer), but in recent years the conviction has been reached by most workers on the subject that no distinction by bacteriological methods is possible. It is, therefore, unnecessary to give a special description of its characters.

Identity with B. influenzæ.

Distinction was at one time thought to be possible on both morphological and cultural grounds (Axenfeld, 1913). The true Koch-Weeks bacillus was described as a rod, whereas *B. influenzæ*, which, it was admitted, could be found in conjunctivitis, was a coccobacillus. It is evident that no such distinction can now be maintained, though it appears to be a fact that the bacilli in epidemic conjunctivitis are usually in the rod form. In culture, the Koch-Weeks bacillus was supposed to grow better on serum or ascites agar than on the blood-agar of Pfeiffer. Such blood-agar, especially when prepared with other than human blood, was said often not to permit of growth at all. It is difficult to say now whether these observations were correct. It is known, on the one hand, that ordinary blood-smeared agar is not an optimal medium for the hæmoglobinophilic bacteria and not seldom leads to failure in culture, and, on the other hand, that small traces of blood in serum or ascites fluid are difficult to avoid and often suffice for their growth. It may be said, at any rate, with a good deal of assurance, that the bacilli, morphologically like *B. koch-weeks*, which have been found in acute conjunctivitis of late years, show no distinguishing feature from the influenza bacillus in cultural behaviour (Davis, 1921[2]; Knorr, 1924; Fildes, 1924). The test for indole-formation divides strains of Koch-Weeks bacillus, like those of the influenza bacillus, into positive and negative groups, and serological investigation, so far as it goes (Knorr, 1924), shows that great diversity of types exists among them just as among different strains of Pfeiffer's bacillus. Moreover, sera prepared with conjunctivitis strains agglutinate strains of the influenza bacillus and vice versa.

On the other hand, conjunctivitis rarely occurs in patients in whose respiratory tract Pfeiffer's bacillus is, nevertheless, present in enormous numbers and obviously pathogenic (though, of course, the same statement holds good for the pneumococcus), and even the severest cases of epidemic Koch-Weeks conjunctivitis are rarely, if ever, associated with respiratory catarrh or other influenza-like symptoms. Hence it is possible that there is a real difference between the two groups; perhaps, more probably, the difference is one of adaptation of particular strains to the conjunctival habitat; in any case, it is safe to say that no differentiation is possible by our present experimental methods except perhaps by direct inoculation of the human eye, which will be discussed below. If successful inoculation

is taken as the criterion, then, as will be seen, the morphological concept of the Koch-Weeks bacillus must be widened to include the cocco-bacillary form.

Pathogenic Action.

In the natural disease, infection is followed by an incubation period of 36 to 48 hours, after which abundant muco-pus appears on the reddened and swollen conjunctiva. In the severer cases a pseudo-membrane may appear as in gonococcal infections. Corneal ulcer is a rarer complication. Constitutional symptoms are almost always absent. Successful experimental infection of the human conjunctiva with pure cultures of the Koch-Weeks bacillus has been recorded by Hoffmann (1900) and by Luerssen (1905) in addition to those already mentioned. The resulting pathological process is said in each case to have been indistinguishable from the ' natural ' disease. Luerssen's (1905) experiment has some points of special interest. He infected one of his own eyes with a morphologically typical Koch-Weeks strain from a case of conjunctivitis, using 1 mgm. of a 24-hour culture, the third generation on pigeon-blood agar. Pus flakes appeared in the conjunctival sac within 6 hours, and at 24 hours the typical disease was present with abundant bacilli in the pus and nasal mucus. After 4 days the eye was treated and returned to normal in about 3 weeks. The other eye had been protected, remained healthy, and was then used for a second experiment with the *B. trachomatis* of Müller, which is now generally regarded as indistinguishable from Pfeiffer's influenza bacillus. In this second experiment he used only one-tenth of the amount of culture (0·1 mgm. of 24-hour, third generation on similar agar, isolated from a case of trachoma), and had only an evanescent reaction ; repeated three days later with a larger dose (0·5 mgm.) the experiment gave a similar result, as also with two other volunteers. The influenza bacillus isolated from a case where acute conjunctivitis was not present thus failed to produce disease. On the other hand, McKee (1907) reproduced the disease with a bacillus isolated from an epidemic of conjunctivitis in Montreal ; the description of this bacillus identifies it as of the cocco-bacillary type of *B. influenzæ*, though McKee himself considered it as different from both *B. koch-weeks* and the influenza bacillus. I have not been able to find records of experimental inoculation of the human conjunctiva with strains of Pfeiffer's bacillus from cases of influenza, but in view of the many negative results with throat and nose inoculation (vide p. 348) success seems unlikely. The conclusion is that for experimental production of conjunctivitis with bacilli of the influenza group strains recently isolated from contagious conjunctivitis must be used. Inoculation into the conjunctiva of other animal species has given uniformly negative result, not only with cultures of the Koch-Weeks bacillus, but with pus direct from an acute case of the disease in man. Hammerschmidt (1921), however, reports success using a mixture of the bacillus with pneumococci ; he states that, on the other hand, influenza bacilli plus pneumococci failed to produce the disease ; his experiments

are worth repeating. With other methods of inoculation the results obtained are apparently identical with those produced by Pfeiffer's bacillus (vide p. 345, and Knorr, 1924), i.e. endotoxic effects alone are obtainable.

Epidemiology.

Epidemics of conjunctivitis in which the pus contains bacilli of the influenza group have been described in all parts of the world, including the tropical and semi-tropical regions (Axenfeld, 1913). In Egypt, the disease appears to be endemic with a curious epidemic rise in the summer months (? fly infection). In temperate climates no seasonal variation can be detected with certainty and the endemic prevalence varies greatly in extent in different places. Children everywhere are chiefly involved, but are said to present the milder forms of the disease ; adults, more rarely infected, more often show the severest types. But, in epidemics, all ages are usually affected and there is little or no evidence of natural immunity. Relapses are frequent, showing that immunity is acquired with difficulty, and the dying down of an epidemic may depend more on diminished virulence of the current strains of the bacillus than on the immunity achieved by the population concerned. Chronic carriers are common among those who have suffered from the disease.

There are no statistics as to the frequency of occurrence of Koch-Weeks bacilli in the normal conjunctiva, but in conjunctivitis, taken generally, the proportion of patients in whom the bacillus can be detected varies from 54 per cent. in Glasgow (Pollock, 1905) to 2·8 per cent. in Copenhagen, in which town the epidemic disease is said not to occur. Bacilli of the influenza group have been found in the conjunctiva as well as in the respiratory tract in measles (Levinthal and Fernbach, 1922).

The relationship of epidemics of conjunctivitis to epidemics of influenza is of peculiar interest in view of the close relationship, if not identity, of the influenza bacilli with the Koch-Weeks bacillus. Hübschmann (1922) and Knorr (1924) are both of opinion that a time relationship exists between the epidemic prevalence of the two diseases, and Fildes (personal communication) has observed the appearance of cases of Koch-Weeks conjunctivitis in greater numbers immediately preceding a wave of influenzal respiratory disease. But it is difficult to believe that these observations are more than coincidental, unless, indeed, one is to suppose that there are two varieties of conjunctivitis, both due to bacilli belonging to the influenza group, but one due to a bacillus which has acquired a special pathogenicity limited to the eye (the Koch-Weeks bacillus), the other due to Pfeiffer's bacillus itself, which has a more general capacity of producing inflammation of mucous membranes ; the latter variety of eye disease might then be, in fact, a precursor and a successor, representing the beginning and the end of the curve of fluctuating virulence of which the peak is an influenza epidemic.

The *B. koch-weeks*, like the influenza bacillus, cannot long resist exposure to conditions outside the human body. Nevertheless, when

protected as it is in disease secretions, it can withstand drying at room temperature for at least 18 hours [silk threads soaked in conjunctival pus (Knorr, 1924)]. In the water of swimming baths the bacilli (from cultures) have been found to survive 3 hours (Dahmann, 1920). Hence the observations of epidemiologists that the principal method of transference of conjunctivitis is by means of handkerchiefs, towels, common wash basins and similar articles, are supported by experimental fact. The special incidence of the disease in schools and barracks is probably to be explained on these grounds; among soldiers, especially, the relative immunity of non-commissioned and commissioned officers observed in Germany during epidemics is said to depend on their special privileges in the private possession of such articles. In tropical countries there can be no question that insect transport is one of the chief factors in spread. Droplet infection is, however, a distinct possibility, since the highly infectious pus enters the nose by the lachrymal ducts, and may be scattered in coughing and sneezing. Swimming baths may convey the infection, and Dahmann (1920) describes an outbreak of Koch-Weeks conjunctivitis of such origin. In other swimming bath outbreaks, however, the conjunctivitis has often been of different type.

Diagnosis.

The diagnosis of the disease is a simple matter; the microscopical appearance of the pus almost suffices, with its crowds of minute Gram-negative rods, many of them intracellular. Culture will confirm this with the appearance on a suitable medium (e.g. Fildes agar) of abundant colonies of typical appearance and characteristic limitation in subculture (failure to grow on ordinary agar). In mild cases and carriers, the use of silk thread soaked in the conjunctival secretion and rubbed on the medium can be recommended. The detection of such subacute and chronic infections, with subsequent isolation and treatment, is probably one of the most useful measures in combating school outbreaks of the disease.

B. hæmoglobinophilus canis.

Historical. This bacillus, the only representative of its class in the influenza group, was first described, under R. Pfeiffer's direction, by Friedberger (1903) who found it in 19 out of 20 adult dogs in a chronic purulent exudate from the preputial sac. Microscopically the pus showed clumps of minute bacilli resembling the influenza bacillus and on cultivation on blood-smeared agar abundant 'dew-drop' colonies appeared. Sub-cultured on ordinary agar or serum-agar the bacillus failed to grow, even in the neighbourhood of staphylococcus colonies, and on blood-agar the giant colonies characteristic of the influenza bacillus when growing along with staphylococci could not be produced. Friedberger rightly concluded that the bacillus was nearly allied to, but distinct from, Pfeiffer's bacillus of influenza. He found that it was absent from the prepuce of younger dogs (4 months and under), and could not be found in bitches; inoculation

with pure cultures failed to infect such animals, and Friedberger's conclusion was that the bacillus was a harmless parasite of the preputial sac and not responsible for the purulent exudate : intratracheal and conjunctival inoculations were also without effect.

Similar observations have since been made by Krage (1910) who found the bacillus in 60 per cent. of dogs, by Kristensen (1922) in Copenhagen (in 9 out of 15 dogs), and by Rivers (1922) in Baltimore.

Morphology. As stated above, the bacillus closely resembles *B. influenzæ ;* in culture it is pleomorphic, both ' coccal ' forms and long rods being found.

Culture. The colonies on ' influenza ' medium resemble those of the influenza bacillus, i.e. on blood-smeared agar dew-drops, on optimal medium translucent colonies of about 2 mm. maximum diameter, i.e. smaller than the typical influenza bacillus. The bacilli are not hæmolytic ; most strains produce indole in abundance. They ferment glucose and grow better on media containing this sugar. Like influenza bacilli they require frequent subcultivation and die out rapidly in the cold. They are ærobic and have the same temperature range for growth as the influenza bacillus.

Distinction from Pfeiffer's bacillus. Rivers (1922) was the first to state clearly that they require only X-factor, i.e. autoclaved hæmatin, for artificial growth on ordinary agar. This explains the observation made both by Friedberger (1903) and by Kristensen (1922) that the bacillus grows better than the influenza bacillus on agar containing minimal amounts of blood or hæmoglobin ; in such a medium there is still ample blood-pigment but insufficient V-factor. Fildes (1923) confirmed this observation of Rivers, and also showed that the independence of V-factor shown by *B. hæmoglobinophilus canis* is due to its power of synthesizing V-factor in a nutrient medium. Kristensen (1922) had already noted that the bacillus does not show the ' satellite ' phenomenon (giant colonies round a staphylococcus growth), but itself produces satellitism with true influenza bacilli—facts which are easily explained on this basis. The serological behaviour of the bacillus has been examined by Odaira (1911). He used one strain (freshly isolated) and found that it was agglutinated by his anti-Pfeiffer sera, while a serum prepared with it agglutinated his Pfeiffer strains. His experiments show, however, that he was working with highly sensitive suspensions, and may, therefore, be unreliable ; repetition of this work is desirable. Kristensen (1922) could not obtain suspensions of his strains sufficiently stable for agglutination tests.

B. para-influenzæ and Hæmolytic Influenza Bacilli.

Historical. The hæmolytic influenza bacilli were first noted by Prichett and Stillman (1919) during bacteriological examination of the throats of influenza patients and healthy persons during the pandemic period ; these authors regarded them as confusing but non-influenzal, and called them ' X ' bacilli. Rivers and Leuschner (1921) classed them

as hæmolytic influenza bacilli. *B. para-influenzæ* was found by Rivers (1922) in two cases of influenza (one in the throat, the other in the trachea *post mortem*): both strains were non-hæmolytic, but otherwise resembled the ' X ' bacilli.

Differentiation. Microscopically the bacilli of this group are usually, but not always, coarser than Pfeiffer's bacillus, and resemble *B. typhosus* in size and shape. Involution forms are common, especially long swollen threads. Many strains are indistinguishable from the third, pleomorphic, group of the true influenza bacillus (vide p. 329), and a few resemble the first cocco-bacillary group. The distinguishing feature of the class is the ability of its members to grow indefinitely in media free from hæmatin (or other source of X-factor) provided that an abundant supply of V-factor (yeast, &c.) is supplied (Fildes, 1924). The hæmolytic strains are distinguishable, in addition, by their power of dissolving blood in a medium (liquid or solid); the clear halo surrounding their colonies on blood-agar (10 per cent. blood mixed with agar) is exactly like that produced by the β type of hæmolytic streptococcus (scarlatinal, &c.). Exceptional strains, however, have less hæmolytic power and a halo of the α type (narrow, not perfectly clear) is produced by them. The hæmolytic power remains constant in subcultures with rare exceptions. In concordance with their dependence on V-factor the bacilli of this group show the satellite, giant-colony, formation on blood-smeared agar in the vicinity of colonies of saprophytic bacteria. Unlike *B. influenzæ*, they will grow in such circumstances even on ordinary nutrient agar, though not in the absence of the saprophyte. Most strains of the group die out much more rapidly on ' influenza ' media than does Pfeiffer's bacillus, five days representing, in general, the limit of their viability; even prolonged subculture on artificial medium does not increase this poor viability (with some exceptions).

Colonies on Fildes agar are distinctly more opaque than those of Pfeiffer's bacillus; ' rough ' colonies are common. In other cultural respects, including the division of the group into positive and negative indole-formers, no distinction can be drawn between the hæmolytic bacilli and *B. influenzæ*.

There are no data as regards serological reactions except one observation (Kristensen, 1922) that 3 strains failed to agglutinate with all of 14 sera agglutinating influenza bacilli.

Distribution. No extensive observations have been made on this question. Pritchett and Stillman (1919) found 24 ' X ' strains out of about 350 cultures from influenza patients and normal persons during the pandemic. Rivers and Leuschner (1921) found hæmolytic influenza bacilli in October, 1919, in 14 of 52 Baltimore medical students, and in 1920 in 13 of 52 influenza patients. Bloomfield (1921) noted that in the same group of persons there were few in the spring of 1920, whereas in the autumn they could be isolated from almost all. This was the converse of his findings of true influenza bacilli, which were present in all in the

spring, and in but few in the autumn. There can be no doubt that these bacilli are fairly common inhabitants of the normal throat.

The influenza-like bacilli isolated from the throat of cats by Rivers and Bayne-Jones (1923) form an interesting addition to the para-influenza group. These were found in 6 out of 50 cats, were non-hæmolytic but strictly dependent on V-factor (yeast extract) for their growth. One strain, in addition, was found which required not only the two growth-factors like *B. influenzæ*, but also unaltered protein for its growth.

Fermentation reactions. The hæmolytic influenza bacilli ferment glucose, saccharose and usually maltose but not lactose, mannitol or dulcitol. Their saccharolytic power is a definite feature distinguishing them from the *B. influenzæ* (Pfeiffer).

Pathogenicity. On inoculation into animals, Pritchett and Stillman (1919) found no pathogenic effect with the hæmolytic strains. There is no record of their isolation in human lesions.

Intermediate strains. Hæmolytic strains requiring both X- and V-factors have been described both by Fildes (1924) and Valentine and Rivers (1927). The latter authors, however, noted that hæmolysis with such strains was feeble and that they resembled in other respects Pfeiffer's bacillus rather than the hæmolytic group. Further studies will no doubt increase the number and range of such intermediate strains.

Bacilli of the Influenza Group in Meningitis, Arthritis, &c.

A characteristic disease of infants consisting of a combination of meningitis, arthritis and septicæmia with influenza-like bacilli in the purulent exudates and in the blood has been described by many observers (Slawyk, 1899; Cohen, 1909; Ritchie, 1910; Henry, 1912; Nabarro and Stallman, 1924, *et al.*). In some infants arthritis alone occurs (Fraser, 1911; Taylor, 1927) with a similar bacteriology; such arthritis cases appear to be fairly common at the present day and are usually not fatal.

The relation of these bacilli to Pfeiffer's bacillus has been much discussed. Cohen (1909) considered that his strains (3), though indistinguishable morphologically and culturally from the *B. influenzæ* of respiratory origin, must be classed apart, since, unlike this bacillus, they produced fatal septicæmia on intravenous inoculation into rabbits.

Obviously this distinction alone is not valid as a means of separation, especially as Cohen and Fitzgerald (1910) admit that some strains of Cohen's bacillus may lack such virulence; in fact, most of the pure arthritis strains are non-virulent for animals (Taylor, 1927). There is no doubt, on the other hand, that many of these meningitis and arthritis strains do differ from the typical Pfeiffer's bacillus in their first subcultures. Morphologically they are often much larger, resembling in size and appearance the *B. typhosus*, and their colonies are distinctly more opaque. But these differences tend to disappear during cultivation. The writer possesses both meningitis and arthritis strains which, originally of typhoid-like appearance, are now indistinguishable from some, at least, of the

strains of respiratory origin. And in their dependence on growth factors these strains begin and remain as much restricted as the *B. influenzæ* itself. Ritchie (1910), it is true, found that some of his meningitis strains after prolonged subculture could grow feebly on hæmoglobin-free agar, which would definitely separate them from the influenza group, but the pitfalls in making such a distinction have been already shown to be too serious for its unquestioning acceptance. It is probable that no separate group is involved in these meningitis and arthritis strains, but that all are derived from ' normal ' hæmophilic inhabitants of the nasopharynx, among which several varieties, hæmolytic and non-hæmolytic, X-dependent and X-independent, have already been shown to exist. There is, therefore, no sufficient reason for classifying them otherwise than as belonging to the influenza group, though, no doubt, further study of the hæmophilic bacilli of the nasopharynx may lead to their identification with varieties from that region and to the discovery of valid distinguishing features.

Bacilli of the Influenza Group in Animals.

Such bacilli in animals have rarely been identified, though hæmophilic bacteria of less restricted growth requirements, e.g. the Pasteurellas, Riemer's Bacillus of goose-septicæmia (Frosch and Bierbaum, 1909), Wolff's (1903) rat bacillus and Marx's (1908) bacillus from a tiger, have been found. But it is possible that more thorough search would reveal the existence in various animals of bacilli to be classed definitely with the *B. influenzæ*. Olsen, quoted by Kristensen (1922), found such bacteria in dogs that had died of distemper and Kristensen himself (1922) found them in the mouth in 5 out of 60 guinea-pigs (microscopically atypical), though he failed to find any in 43 mice and 33 horses.

Relationship of the Influenza Group to other Bacteria.

It is difficult to perceive affinities between the influenza group and other bacterial species. Its position is sharply marked off by the dependence of its principal member, the bacillus of Pfeiffer, on the ' peroxidase ' factor, for which no analogy is known among other bacteria. A bridge may exist between those members of the group independent of X-factor, i.e. the hæmolytic influenza bacilli and *B. para-influenzæ*, and other species which may display optimal growth only in presence of substances approximating in quality to the V-factor ; but no species is yet known in which this dependence is so absolute and permanent. [*B. pertussis* (vide p. 396), mistakenly supposed to have hæmophilic characters on first isolation, probably owes its optimal growth on blood-containing media entirely to its demand for unsplit protein, a demand which rapidly lessens on cultivation on peptone-containing media ; even on first isolation it requires neither hæmatin nor added V-factor for growth].

No indication of the evolutionary origin of the influenza group is, in fact, available in the state of present knowledge.

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Bacterium Pneumosintes (Dialister pneumosintes).

By J. E. McCartney.

Olitsky and Gates (1921[1-5], 1922[1-5], 1923) described the isolation of a small micro-organism, which they called *Bacterium pneumosintes*, from the filtered nasopharyngeal secretion of patients suffering from epidemic influenza. Similar organisms were isolated from the lung tissue of rabbits inoculated intratracheally with nasopharyngeal washings from influenza patients. Several observers have previously reported the isolation of small micro-organisms from epidemic influenza (see p. 354).

The method of isolation of the organism by Olitsky and Gates was as follows: Saline washings from the nose and throat were obtained from early cases of typical epidemic influenza within the first 36 hours of the

disease. Amounts of 3 c.cm. were injected intratracheally under ether anæsthesia into healthy rabbits. The animals showed within 24 to 48 hours a rise of temperature and a marked lowering of the number of leucocytes, the fall being due to a reduction in the number of mononuclear cells. This reaction is similar to that in human influenza. If the rabbits are killed at this stage, the lungs are found to be congested and œdematous, but there is no consolidation. Portions of the lung are incubated at 37° C. in Smith-Noguchi medium. After 5 to 8 days' incubation there occurs in favourable circumstances a faint clouding of the medium around the kidney tissue. Microscopical examination after staining with well-ripened Loeffler's methylene blue reveals numerous small bacilloid bodies 0·15 to 0·3μ long. Identical bodies are obtained when Berkefeld V or N filtrates of nasopharyngeal secretion from early cases of influenza are similarly cultivated. Olitsky and Gates assume that the role of this organism is to lower the resistance of the respiratory tissue, rendering it liable to attack by secondary invading organisms. The latter produce the pulmonary complications which are responsible for the high mortality of the disease. They, therefore, named the organism *B. pneumosintes* from πνεύμων lung, and σὶντης injurer or devastator.

Morphology and Staining Reactions.

B. pneumosintes is a rod-shaped organism two or three times longer than broad. The long axis measures 0·15 to 0·3μ. Occasionally larger individuals are seen, but the organism has little tendency to pleomorphism. No granules, clubs, spores, flagella, capsules, or involution forms have been noted, and the organism is non-motile. Cultures in the eighteenth generation are morphologically identical with the initial culture. Well-ripened Loeffler's alkaline methylene blue is the most satisfactory stain.

The organisms are most numerous in the bottom of the Smith-Noguchi tube around the kidney fragment. Films are made in the usual manner, flooded with the methylene blue, and steamed gently over a flame for two minutes. Films thus prepared from typical cultures show minute bodies of regular morphology, stained deep purple, and clearly differentiated from the background of pale blue protein precipitate. The bodies are very numerous in culture. Usually solitary, they are sometimes found in diplo form, and occasionally in short chains of 3 or 4 individuals. In older cultures, especially, clumps may be found, particularly in the region of the kidney tissue. They can readily be distinguished from the small protein particles present in the medium, but a stain which tends to precipitate obscures the individual bacteria. For this reason Giemsa's stain or carbol-fuchsine are unsuitable. *B. pneumosintes* is uniformly and constantly Gram-negative.

Examination by dark-ground illumination is unsatisfactory on account of the similarity in size between the organism and the numerous small particles showing Brownian movement in control preparations.

Cultivation.

B. pneumosintes, in primary and early subcultures, is a strict anaerobe, and requires the use of Smith-Noguchi medium (Vol. IX). Well-established strains, however, can grow under partial oxygen tension. One c.cm. of suspected fluid or 0·5 c.cm. of emulsion of affected lung tissue is used as the inoculum. The tubes are incubated at 37° C. for a week to 10 days.

In primary cultures growth is evidenced about the fifth day by a faint haze in the medium, extending upwards about 1 cm. from the kidney tissue. This becomes denser and reaches its maximum at about the eighth day. On standing, the cloud settles in about two weeks, leaving the fluid above the kidney tissue clear. In cultures of established strains, growth is observed about the third day, and the maximum cloudiness is reached at the fifth day.

On rabbit-blood agar plates after 7 to 10 days' incubation at 37° C. in the anaerobic jar, *B. pneumosintes* grows as minute colonies which require a low-power microscope for their definition. They are round, raised and convex with a smooth edge and a colourless translucency. They exhibit no characteristic feature, and do not alter or discolour the medium. Colonies tend to remain discrete even when close together, but with heavy inoculations they may coalesce to form raised plaques of confluent growth. In old-established strains, even after three years' cultivation *in vitro*, the organism will not grow in autoclaved media without fresh tissue or body fluids. The ascitic fluid, however, may be replaced by nutrient bouillon ; and fresh defibrinated blood, vegetable tissue, or the products of bacterial metabolism, may be substituted for rabbit kidney. Avery and Morgan (1921) find that *B. pneumosintes* grows readily in a simple infusion broth containing fresh vegetable tissue such as potato, turnip, and parsnip.

B. pneumosintes may be grown in culture in symbiosis with other organisms such as staphylococci and streptococci. Olitsky and Gates showed that if the Smith-Noguchi medium is enclosed in a dialysing membrane such as a collodion sac, and surrounded by distilled water or saline solution, anaerobic conditions are soon established throughout the system, and nutritive and growth-promoting substances of the medium diffuse through the membrane in sufficient quantities to support a luxuriant growth in the surrounding liquid. The protein precipitate that collects round the kidney tissue is thus retained within the sac, and the organism obtained in the fluid outside free from protein particles.

Biochemistry.

B. pneumosintes ferments glucose, producing acid but not gas. The limiting hydrogen-ion concentration thus produced is pH 5·2 to 5·3. After growth has apparently ceased, organisms may remain viable in the acid medium for several days.

Filterability.

According to Olitsky and Gates *B. pneumosintes* passes Berkefeld V and N filters, whether from human nasopharyngeal secretion, in cultures, or in the emulsified lung tissue of infected rabbits. Pfeiffer (1925), however, quotes Prausnitz, who was unable to pass a culture sent him by Olitsky through Berkefeld filters.

Distribution.

The organism has been isolated from the nasopharyngeal secretions of influenza patients in the early stages, i.e. within the first 36 hours of the disease. It has not been isolated from individuals not suffering from influenza, nor in influenza patients in the later stages of the disease. Olitsky and McCartney (1923) examined cases of common cold, using cultural methods similar to those of Olitsky and Gates, and did not find *B. pneumosintes* in any of the cultures from the cases, both normal and with colds, that were studied. Holman and Krock (1923[1 & 2]) have described a minute anaerobic coccus present in the respiratory tract in patients suffering from tonsillitis and tracheitis, and also in the oral secretion of normal persons and rabbits. This organism, named *Staphylococcus parvulus*, which appears to be identical with the *Micrococcus gazogenes alcalescens* of Lewkowicz, produces gas in cultures, thereby distinguishing it from *B. pneumosintes*. A similar organism was obtained by Thomson (1925) from cases of measles, scarlet fever, and from normal persons. Thomson states that it is different morphologically and culturally from *B. pneumosintes*. The latter is present in the lung tissue of rabbits inoculated intratracheally with it from recently isolated cultures, and from whole and filtered nasopharyngeal washings from early cases of influenza. Microscopically, it may be seen deep in the ciliary margin of the bronchial epithelium, and occasionally in alveolar cells.

Pathogenic Action.

In Animals.

Recently isolated cultures give rise to definite pathological lesions in the rabbit. When injected intratracheally in amounts of about 3 c.cm., the animals usually show 24 hours after inoculation some rise in temperature, and a conjunctivitis which may vary from a simple congestion of the palpebral conjunctiva to marked injection of both ocular and palpebral conjunctivæ. There is a definite and often marked leucopenia, the number of lymphocytes being considerably reduced. These conditions persist for 2 or 3 days, when the animal returns to normal. If the rabbit is killed during the reaction, the lungs are found to be voluminous and emphysematous. Numerous hæmorrhages are seen on the surface, both diffuse and discrete, and often in the form of minute petechiæ. The pleura is not involved. On section the cut surface drips a frothy

blood-stained fluid, and hæmorrhages are scattered throughout the parenchyma. The bronchi and trachea show a muco-purulent exudate on a congested and exfoliated epithelium.

Microscopic examination of the lungs shows numerous hæmorrhages, while the alveolar walls are distended by œdema and disrupted by the emphysema. In the alveoli there is a cellular exudate consisting of mononuclear cells and some polymorphonuclear cells. The lung capillaries are distended with blood. No pneumonic consolidation is present. The bronchi show thick congested walls with the lumen partly filled with red blood cells, leucocytes and fragments of exfoliated or necrotic epithelium.

Many of the lesions above described, particularly the hæmorrhage, are said by Maitland, Cowan and Detweiler (1920) to be due to the trauma occasioned during the killing of the rabbits by hitting them on the back of the head. If no violence is used in killing the animals the lesions described by Olitsky and Gates are stated not to occur.

Guinea-pigs are similarly susceptible to injection.

When the organism has been maintained in artificial subculture over many generations it loses its pathogenicity for animals.

Rabbits injected intratracheally with *B. pneumosintes* exhibit a much lessened resistance of the lung tissue to invasion by other bacteria. Thus, in one series of experiments by Olitsky and Gates it was regularly found that three million pneumococci of an atypical Type II strain, when injected into rabbits, rapidly disappeared from the blood-stream and produced no lesions. If this dose of pneumococci was similarly injected the day after intratracheal inoculation with *B. pneumosintes*, pneumococcal septicæmia and pneumonia resulted. It is assumed that the respiratory and pulmonary complications in human cases of influenza are due to secondary organisms such as pneumococcus, streptococcus, *B. influenzæ* (Pfeiffer) invading tissue previously damaged by *B. pneumosintes*.

In Man.

Lister (1922) obtained a filter-passing organism from early cases of influenza, and carried out experiments on human volunteers. Sixty c.cm. of a culture in the second generation were divided into three portions, one-third was placed in a spraying bottle, one-third passed through a Berkefeld filter, and placed in a second bottle, while the remaining third was heated to 56° C. for 30 minutes, and thereafter placed in a third bottle. Six volunteers received the unaltered culture, 7 the filtrate, and 6 the heated filtrate, about 1·5 c.cm. being sprayed into the nose and throat of each person. Only 1 of these 19 volunteers developed a typical attack of influenza, which commenced 19 hours after spraying with the untreated culture. Minute organisms were observed in the smears of the nasal secretion, and cultures of the filtered nasal washings in Smith-Noguchi medium showed an abundant growth. Two other persons who had received the unaltered culture showed a slight rise of temperature, and one of them showed a marked leucopenia. These results, while

suggestive, do not prove that the minute filter-passing organism is the cause of influenza. Pfeiffer (1925) quotes an experiment of Prausnitz who sprayed himself with a culture of *B. pneumosintes* without giving rise to any symptoms. This culture was from an established strain sent by Olitsky, and it is possible that the organism had lost its pathogenicity.

Resistance.

B. pneumosintes is readily destroyed by heat and by antiseptics. It is killed when cultures are heated to 56° C. for 30 minutes or exposed to chloroform vapour for an hour. When present in affected lung tissue of the rabbit, it withstands the action of 50 per cent. glycerol for periods up to 9 months. Cultures in Smith-Noguchi medium may remain viable at room temperature for periods up to $2\frac{1}{2}$ years or more. It withstands freezing and drying *in vacuo*, and in this condition may be viable for at least 2 months. Stock cultures in blood broth also remain alive for many months.

Immunity.

Rabbits injected intratracheally with cultures of *B. pneumosintes* and allowed to recover, do not react, as evidenced by leucopenia with diminution of lymphocytes, to subsequent injections. Rabbits inoculated intravenously with the living organism from cultures in *B. coli*-treated broth develop agglutinins, precipitins, complement-fixing antibodies and opsonins. Agglutination occurs in dilutions up to 1 in 160.

Rabbits immunized with successive amounts of killed organisms develop specific agglutinins in many instances (11 out of 15), although some fail to do so. The animals thus treated show resistance to subsequent intratracheal injection with living recently isolated organisms, whereas control animals react in the usual manner. Similarly, infective rabbit lung tissue fails to evoke reactions in immunized rabbits, whereas normal control animals show no such resistance to infection.

Human serum from recovered cases of epidemic influenza shows in some instances specific agglutinins. Agglutination, however, is usually only obtained in low dilutions such as 1 : 5 or 1 : 10, and when the organism is suspended in buffer solution of pH 6·3 in order to render it more sensitive to agglutination. Sera of normal persons do not cause agglutination in the above-mentioned dilutions. In some instances precipitins can also be demonstrated in serum from recovered influenza cases. Antibodies have been demonstrated up to 5 months after recovery from the disease.

Relation to Epidemic Influenza.

Data. A number of healthy men from the United States Army volunteered to submit to inoculation with killed cultures of *B. pneumosintes*. The vaccines were standardized so that the opacity of the bacterial emulsion was equal to that of 1,000 million staphylococci per c.cm.

Three injections of 0·5, 1·0, and 2·0 c.cm. respectively were administered. No severe local or general reactions were noted, the effects being milder than those experienced after antityphoid vaccine. On the tenth or eleventh day after final injection the serum of 7 among 9 men examined showed the presence in low dilutions of agglutinins. A subsequent outbreak of influenza, however, demonstrated that the injections had afforded no protection against influenza, that the inoculated volunteers were as liable to infection, and that the disease was as severe in them, as in uninoculated controls.

Attempts by other workers to confirm the results of Olitsky and Gates, and to isolate *B. pneumosintes*, have usually been unsuccessful. Certain observers, however, have reported successful isolation. The failure may be due to the lack of suitable cases, or inability to secure them in the early stages of the disease.

Olitsky and Gates (1922[4]), in the 1922 epidemic of New York, obtained four new strains which were identical in every respect with those previously isolated by them. Lister (1922) obtained cultures of an organism apparently identical with *B. pneumosintes* after culture of filtered nasal washings in Smith-Noguchi medium. Culture was successful in 5 out of 15 cases. Experiments with these cultures on man have already been referred to.

Loewe and Zeman (1921), in New York, stated that they obtained consistently from filtered nasopharyngeal washings of early influenza cases organisms resembling *B. pneumosintes*, and these when injected into experimental animals (Baehr and Loewe, 1922) produced a characteristic clinical and pathological picture.

Olitsky and McCartney (1923[2]) obtained *B. pneumosintes* in 4 out of 6 cases examined. One strain was obtained direct from culture in Smith-Noguchi medium of Berkefeld V filtrates of nasopharyngeal washings, while the other three strains were isolated from the lung tissue of rabbits injected intratracheally with the whole washings. All the strains were identified as agreeing with those isolated by Olitsky and Gates in the 1918–19, 1920, and 1922 epidemics, in morphology, cultural properties, animal effects, and serological reactions with antipneumosintes serum. Agglutinins in serum dilutions varying from 1 : 2 to 1 : 10 were demonstrated in 6 of 10 patients recovered from influenza.

Nakajima (1923) in Tokio cultivated two strains from pharyngeal washings, and one strain from the lung tissue of a fatal case of influenza. Hall (1926) also confirmed the work of Olitsky and Gates, while Gates (1926) isolated two new strains from early influenza cases. Branham (1927) found organisms resembling *B. pneumosintes* in cultures of filtered nasal washings of early influenza cases.

Discussion. It cannot be regarded as proved that *B. pneumosintes* is the ætiological agent of epidemic influenza. It is not found consistently in all cases of influenza, and examination of typical early cases of the disease, under the optimum conditions, may fail to reveal its presence. The lesions produced by *B. pneumosintes* in animals are not typical of the

human disease, and the pathological findings in the lungs, slight as they are even when most pronounced, may be due to the technique of injecting and killing the animal. Pfeiffer (1925) quotes Hottinger, who found in rabbits after intratracheal inoculation with sodium chloride solution the same changes as those described by Olitsky and Gates. The demonstration of antibodies in recovered persons is not wholly convincing, and the failure of vaccines to protect against influenza also tends to negative any ætiological relationship to the disease.

Comparatively few workers have succeeded in isolating *B. pneumosintes*, while many competent observers have failed completely to confirm the results of Olitsky and Gates. There can be no doubt, however, that *B. pneumosintes* is a definite living multiplying organism. Its morphological, cultural, and biological properties are indisputable. It has been found only in early cases of influenza, and has not been isolated from any other inflammatory conditions of the respiratory tract.

The writer has had the opportunity of working with Olitsky and Gates in the Rockefeller Institute in New York, and, with Olitsky, has isolated *B. pneumosintes* from 4 of 6 cases of early typical uncomplicated influenza. As the work was technically difficult, it is understandable why other workers have failed in their attempts.

The experimental production by Lister (1922) of influenza in a volunteer by spraying the nose and throat with a culture of *B. pneumosintes* affords evidence, not, however, amounting to proof, that this organism is the incitant of the disease.

In conclusion, it may be stated that the available evidence is not sufficient either to prove or to disprove any ætiological relationship between *B. pneumosintes* and epidemic influenza, but the work up to the present shows that this organism is in some way related to the disease.

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CHAPTER VII. WHOOPING COUGH AND *BACILLUS PERTUSSIS*—CHANCROID AND *BACILLUS DUCREYII*—CONJUNCTIVITIS, *BACILLUS LACUNATUS* AND OTHER ORGANISMS.

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Introduction.

The three principal organisms dealt with in this chapter, viz. *Bacillus pertussis* of whooping cough, *Bacillus ducreyii* of soft chancre and *Bacillus lacunatus* of a form of conjunctivitis, are grouped together for convenience, rather than because they are related to one another. It is true they are included, with *Bacillus influenzæ*, in the genus '*Hemophilus*' of the Society of American Bacteriologists, but as Fildes (1923) points out they are probably not strictly hæmoglobinophilic, though preferring a culture medium containing blood, blood-serum, or ascitic fluid.

In order to render the subject of 'conjunctivitis' more complete, some other organisms receive brief notice.

Whooping Cough.

Ætiology.

The search for a parasitic cause of whooping cough was begun long ago, and so far back as 1870 Letzerich described the presence of a fungus in the disease. Burger (1883) published the next paper of importance, in which he described (with two woodcuts) the presence of large numbers of small ellipsoidal rods in stained films of the expectoration. The description and illustrations which he gives suggest that he actually saw the *Bacillus pertussis*. From then onwards various bacterial forms and also amœboid protozoa were described. Afanassjew in 1887 described a bacillus, and important papers by Czaplewski and Hensel and by Koplik were published in 1897. It is not unlikely that Koplik saw and cultivated the organism we now know as *Bacillus pertussis*, for he obtained pearly colonies of a small bacillus on an ascitic-agar medium, but his cultures were impure and probably contained *Bacillus influenzæ* and other organisms.

In 1901 Jochmann and Krause described a small bacillus which they had isolated on blood-agar from the sputum. It formed dew-drop colonies and was very like the influenza bacillus, which it probably was. They named their organism '*Bacillus pertussis*, Eppendorf' (from Hamburg-Eppendorf, where the hospital was), and this appears to be the first use of the present Latin name for the organism of whooping cough.

In 1906, Bordet and Gengou announced that they had succeeded in isolating from whooping-cough patients by the use of a special potato-blood-agar culture medium a small and distinctive bacillus, and they adduced considerable evidence of its causal relation to the disease. This work was confirmed by them in 1907, and by Fraenkel in 1908, and Klimenko in 1909, and later by many other observers.

The organism isolated by Bordet and Gengou, the *Bacillus pertussis*, is now commonly, though perhaps not universally, accepted as being the causative organism of whooping cough.

Bacillus pertussis.

Morphology.

Bacillus pertussis (Bordet-Gengou bacillus, *Hemophilus pertussis*) is an organism which in primary culture grows best in a culture medium containing fresh blood, and for this reason it has been classed with the hæmophilic bacteria. It bears considerable resemblance to *B. influenzæ*, with which it is frequently associated in the whooping-cough patient.

In the sputum of the early stages of the disease *B. pertussis* is present in large numbers, particularly in the viscid pellets voided at the end of a paroxysm of coughing. It is a small rod, ovoid in form, measuring 1·0 to 1·5μ in length, by 0·3 to 0·5μ in breadth. It is distributed without order among the cells of the exudate, and is sometimes phagocytosed by some of the cells. The majority of the organisms occur singly, occasionally they may be seen in pairs end to end, but chains, even short ones, do not occur. There is slight variation in size among the bacilli as a whole, but a few are a little longer, others a little shorter and almost coccoid.

B. pertussis is Gram-negative and is best stained with phenol-methylene blue or with phenol-toluidine blue. In the stained film, the contour and poles of the organism tend to stain more deeply than the centre. The longer individuals often show a blue point in the cytoplasm, as though chambered. With toluidine blue staining, the general tint assumed is a lilac one (Giese, 1918).

In pure culture on a solid medium, e.g. blood-agar, *B. pertussis* has much the same morphology and arrangement as in the sputum, though pairs of organisms are perhaps more frequent. In fluid culture media, likewise, it maintains its size and form, and is generally arranged in groups, though short chains of three or four elements may occur. Involution forms are very infrequent, and long filaments are never met with. In the latter respect the organism differs from *B. influenzæ*.

B. pertussis is non-motile, is not encapsuled, and does not form spores.

Cultivation.

B. pertussis is markedly aerobic and grows only at 37° C. or thereabouts. In primary culture it is somewhat difficult to grow, and it does not develop on nutrient agar and gelatin, inspissated blood-serum, and in nutrient broth. By repeated passages on a medium containing

less and less blood, it becomes acclimatized, and capable of developing on nutrient agar without blood or serum.

Bordet and Gengou owed much of their success in the investigation of the organism to the discovery of a culture medium, potato-blood-agar, upon which *B. pertussis* grows with considerable readiness, and yields somewhat characteristic colonies and growths. This medium, or some modification of it, has been employed in most subsequent investigations. (For method of preparation, see Vol. IX.)

The colonies of *B. pertussis* appear on this medium after 48 hours' incubation at 37° C. as small points, which increase in size up to 1 mm. in diameter during the next 48 hours, and at the end of this time form round, sharply defined, moist, raised, opaque colonies of a pearly-white lustre, surrounded by a zone of hæmolysis. *B. influenzæ* also grows well on the Bordet-Gengou medium, and is frequently associated with *B. pertussis* in the primary cultures, but its colonies are thinner and less raised, and are more diaphanous particularly at the edges, than the *pertussis* colonies, are bluish or greyish in appearance, and are not surrounded by a hæmolytic zone.

B. pertussis will also grow on ascitic-fluid agar, on serum-agar, and on nutrient agar smeared with blood, and will develop in a glycerin nutrient broth containing blood, blood-serum, or ascitic-fluid.

Viability of cultures is poor unless they be kept in cold storage; with this precaution potato-blood-agar cultures will remain alive for three or four weeks.

The method for primary isolation from the patient will be found under 'Diagnosis', p. 404.

As *Bacillus influenzæ* is so frequently associated with *Bacillus pertussis*, the differential table, Table I (Madsen, 1925), may be of service:

TABLE I.

B. pertussis.	*B. influenzæ.*
MORPHOLOGY.	
Fairly regularly ovoid. The bacilli vary little throughout the entire preparation.	Rarely showing the ovoid form of, and more pleomorphous than, *B. pertussis.*
GROWTH ON GLYCERIN-POTATO-BLOOD-AGAR.	
Slow; colonies never visible macroscopically in 24 hours.	Rapid; colonies 1–2 mm. after 24 hours.
APPEARANCE OF THE COLONIES.	
Raised, opaque, somewhat resembling drops of mercury, or very white and then often flat.	Flat, thin and transparent.
HÆMOLYSIS.	
Well marked after two or three days. Boundary not well defined.	None.
GROWTH ON ASCITIC AGAR (HÆMOGLOBIN-FREE).	
Excellent, even with recently isolated strains.	No growth as a rule; exceptionally a very scanty growth.

Biochemical Reactions.

B. pertussis possesses little chemical activity. It is not proteolytic and never liquefies blood-serum. Its fermentative power is also very small. Stillman and Bourn (1920) state that it produces a small amount of alkali in glucose and lactose media, but has no action on galactose, lævulose, maltose, saccharose, mannitol, dextrin and inulin. It forms no indole and does not reduce nitrates. Ferry and Noble (1918) state that, after repeated transplantations, *B. pertussis* renders litmus milk alkaline and gives a tan-coloured growth on potato.

Immunological Reactions.

B. pertussis forms no exotoxin, and according to Bordet and Gengou (1909) injection of the endotoxin into animals is not followed by the production of an anti-endotoxic serum. Injection of the organisms into an animal is followed by the formation of agglutinins and of complement-fixing substances.

Bordet and Sleeswyk (1910) stated that they could influence the antigenic properties of *B. pertussis* by the use of different culture media. Thus, if the organism were grown on a blood medium, e.g. the Bordet-Gengou medium, and rabbits or horses were immunized with this culture, the resulting agglutinins proved active upon the bacillus grown on the blood medium, but less active upon the bacillus grown on nutrient agar. Moreover, the agglutinin was completely absorbed from such a serum by saturation with bacilli grown on the blood medium, but was not absorbed by a strain grown on agar. Similarly, if a strain grown on agar were used to produce an agglutinating serum, the results were reversed. When, however, an agar strain was grown on a blood medium, it assumed the antigenic characters of the strain grown continuously on the blood medium. These observations were not, however, confirmed by Krumwiede *et al.*, nor by Povitzky and Worth (1916), though the latter found that a horse inoculated with strains grown on the Bordet-Gengou medium yielded a serum during the earlier stages of immunization which agglutinated cultures grown on the same medium, but did not agglutinate those grown on a chocolate-agar medium. After a longer period of immunization, this difference disappeared.

The occurrence of agglutination and complement fixation with patients' serum, and the value of these reactions for diagnosis will be dealt with on pp. 403 and 404 respectively.

Agglutination and Types of Bacillus pertussis.

Bordet and Gengou (1907) obtained a good agglutinating serum for *B. pertussis* by inoculating a horse about fifteen times, mostly subcutaneously, with a 48-hours blood-agar slope culture of the organism emulsified in 2 to 3 c.cm. of saline. They noted that agglutination varied in extent with different cultures, and surmised that different strains of the organism may exist. All observations since that date agree that active

and specific agglutinating sera are readily prepared by the inoculation of animals with cultures of *B. pertussis.*

For the preparation of agglutinating sera, Povitzky and Worth (1916) employed rabbits and horses, which yielded better sera than goats and sheep, and the sera obtained had a titre of from 1 : 4,000 to 1 : 10,000. They noted that strains of *B. pertussis* vary in their agglutinability, and they remark that special care is necessary to obtain homogeneous emulsions of the organism for agglutination tests, and that some strains tend to undergo spontaneous agglutination. They consider that agglutination is a more specific test for *B. pertussis* than complement fixation, and in no instance was agglutination of a heterologous organism obtained with *pertussis* serum in higher dilution than 1 : 40. *B. influenzæ* sera sometimes agglutinated *B. pertussis* in a dilution of 1 : 40, but not higher, but four *B. bronchisepticus* sera did not agglutinate *B. pertussis.* Ferry and Noble (1918), however, found that *B. bronchisepticus* serum (titre, 1 : 6,000) agglutinated *B. pertussis* up to a dilution of 1 : 2,000.

Krumwiede, Mishulow, and Oldenbusch (1923) determined the existence of two ' types ' of *B. pertussis* by agglutination tests. The agglutinating serum was prepared by inoculating rabbits intravenously or intraperitoneally on three successive days, followed by more daily injections after an interval of four days without inoculation ; as a rule five injections in all sufficed. Increasing doses of the organism were given, commencing with one-tenth of a ' chocolate ' agar slope culture, and finishing with a whole slope. The antigen employed consisted of the growth from a 48-hours chocolate-agar culture suspended in saline. The suspension was shaken by hand, filtered through cotton wool, standardized to an arbitrary standard of opacity, and preserved by the addition of 0·1 per cent. of formalin (40 per cent. formaldehyde). The sera had a titre of 800 to 2,000, and the tests were carried out by a macroscopic method, the tubes being incubated at 56° C. for 18 hours, and then read.

Two types, A and B, of *B. pertussis* were thus differentiated. Type A antiserum agglutinates type A bacillus to full titre, and also type B bacillus to a considerable degree. Type B antiserum agglutinates type B bacillus to full titre, but does not agglutinate type A bacillus. Type B serum saturated with type A bacillus still agglutinates type B bacillus to the full original titre, but saturation of type A serum with type B bacillus reduces the titre of agglutination for type A bacillus. Nine strains of type A, and ten of type B, were identified.

Hayano in Japan (1923), who had previously noticed differences in the extent of agglutination of different strains of *B. pertussis* by different sera, defines four types by agglutination. The four types also showed slight differences in fermentation reactions.

The question of the existence of various types of *B. pertussis* is one that deserves further investigation, for it may prove of importance in the application of vaccine and serum therapy.

Complement Fixation.

Complement fixation frequently succeeds with patients' serum in the later stages of the disease; this will be dealt with on p. 404. Complement fixation can also be obtained with the serum of animals immunized with cultures of *B. pertussis*. Thus, Wollstein (1909) found that the serum of rabbits that had been injected with cultures for the preparation of an agglutinating serum gave very distinct and striking complement fixation. A good antigen for complement fixation may be prepared by suspending the growth from a 48-hours blood-agar culture of *B. pertussis* in distilled water, shaking the suspension for 24 hours, and finally incubating the suspension at 48° C. for 24 hours (Huenekens, 1917).

Ferry and Noble (1918) call attention to the similarity between a repeatedly transplanted *B. pertussis* and *B. bronchisepticus* in some of their cultural and serological reactions. Both give a tan-coloured growth on potato, and alkali in litmus milk and litmus lactose agar; with *B. pertussis*, however, the changes and growth are slower in developing. *B. bronchisepticus* antiserum agglutinates itself and also *B. pertussis*, but *B. pertussis* antiserum agglutinates only itself and not *B. bronchisepticus*. When *B. bronchisepticus* serum is absorbed with *B. bronchisepticus*, the agglutinins for *B. bronchisepticus* are removed, but not those for *B. pertussis;* and when it is absorbed with *B. pertussis*, agglutinins for *B. pertussis* are removed but not those for *B. bronchisepticus*. The immune serum of either organism fixes complement whichever organism is used as antigen (Ferry and Klix, 1918).

Olmstead and Povitzky (1915–16) found also that immune sera of some *B. influenzæ* strains cross-fixed with *B. pertussis*.

Pathogenic Action of *Bacillus pertussis*.

B. pertussis possesses feeble pathogenic power except for man. Cultures injected in large doses subcutaneously, intraperitoneally, or intravenously into laboratory animals cause death, as was first noted by Bordet and Gengou (1906 and 1907). This was confirmed by Klimenko (1909), who found that the guinea-pig was killed by $1\frac{1}{2}$ to 2 blood-agar slopes, the rabbit by 3 to 4 slopes, and the mouse by $\frac{1}{4}$ slope, injected intraperitoneally. Wollstein (1909) found that $\frac{1}{2}$ to 1 blood-agar slope, subcutaneously or intraperitoneally, did not kill a guinea-pig, but that 2 slopes injected intraperitoneally or intravenously, killed the rabbit.

B. pertussis forms no exotoxin, but an endotoxin can be obtained from cultures, and the death of animals after inoculation with them is apparently due to this agent. Bordet and Gengou noted that the death of an animal after inoculation was not accompanied by any multiplication of the organisms introduced, and Klimenko observed that large doses of dead culture, killed by heating to 60° C., killed animals in the same manner as living cultures in similar doses. Bordet and Gengou (1909)

afterwards prepared this endotoxin by the following method: The growth on fifteen tubes of three-day-old cultures on potato-blood-agar was washed off with 20 c.cm. of 0·6 per cent. saline. The emulsion was evaporated to dryness *in vacuo* at 37° C. over solid caustic potash. The dry residue was ground with 330 mgm. of sodium chloride (dry and sterile). A total of about 630 mgm. of dry powder was thus obtained. The powder was dissolved in 60 c.cm. of distilled water, yielding a solution of the endotoxin in 0·75 per cent. saline; this was allowed to stand for twenty-four hours on ice and then centrifuged.

This solution injected in doses of $\frac{1}{4}$ to $\frac{1}{2}$ c.cm. intraperitoneally into guinea-pigs kills with lesions the same as those produced by the living organism, viz. abundant hæmorrhagic exudate within, and petechiæ on, the peritoneum; intense congestion of the intestines; and copious pleural effusion. Intravenous injection into rabbits kills in eighteen hours with hepatic and renal congestion, and hæmorrhagic foci in the adrenals. Large doses of this endotoxic solution are required to kill by subcutaneous inoculation, but smaller doses which do not kill, e.g. 0·2 c.cm., cause a hæmorrhagic œdema at the site of inoculation, followed by necrosis. Heating the endotoxin solution to 55° C. for half an hour, or treatment with chloroform, toluol, thymol, and particularly with alcohol, destroy most of its activity, and filtration through a Pasteur-Chamberland filter-candle acts similarly. The serum of a horse treated with repeated doses of the endotoxin possessed no neutralizing power for the endotoxin.

In man there are no specific gross lesions. Some laryngitis and bronchial catarrh are generally associated with the disease, and bronchitis and broncho-pneumonia often supervene, caused frequently by secondary infecting agents. Bleeding from the nose and sub-conjunctival and other hæmorrhages are common, due to the strain of coughing. An interesting observation on the histology of the air passages was made by Mallory and Hornor (1912). They found numbers of minute bacteria present between the cilia of many of the cells lining the trachea, bronchi and bronchioles. The organisms usually extended down to the base of the cilia, though sometimes only part way, and frequently caused lateral spreading or 'mushrooming' of the cilia covering a single cell. In many places the cilia were reduced to short stubs or were entirely wanting. The organism was a minute ovoid bacillus, Gram-negative and staining lightly, and strongly suggestive of the Bordet-Gengou bacillus. Mallory and Hornor suggest that the presence of the bacteria mechanically interferes with the action of the cilia and probably leads to their destruction, the result being that normal removal of secretion is prevented. The bacteria and secretion produce a continuous irritation which results in coughing and usually also in the characteristic spasm known as whooping. The viscid pellets voided towards the end of the paroxysm of coughing would therefore consist of the stagnant secretion which has at last been displaced and

expelled. The continuance of the cough long after the *B. pertussis* has disappeared from the patient, who is otherwise well, might be explained by the persistence of the damage to the ciliated epithelium, the regeneration of which is tardy. Mallory, Hornor, and Henderson (1913) afterwards described lesions, similar to those found in man, produced in animals—dog, rabbit, and monkey—by the inoculation of sputum, or of pure culture of *B. pertussis*, into the trachea, even when the animals showed no symptoms, and the organism could frequently be recovered from the respiratory tract. L. W. Smith (1927) found peri-bronchial thickening and enlargement of the tracheo-bronchial lymph nodes present in eight infants suffering from whooping cough and dying from pneumonic complications. Organisms resembling *B. pertussis* were present in the lungs in large numbers in some of the cases, and in seven of the cases *B. pertussis* was cultivated from the lungs.

Feyrter (1927) in an examination of over a hundred cases of whooping cough which came to *post mortem*, found that the bronchial stem is involved early, with the presence of endo-bronchitis and peri-bronchitis and peri-bronchiolitis. The exudation is mostly a leucocytic one, rarely fibrinous and then only to a small extent. Lobular pneumonia is common, but lobar pneumonia is very exceptional. The changes which are found are generally a mixture of younger and older lesions.

Rhea (1915) has directed attention to the similarity of the tracheal and bronchial lesions of whooping cough and of *B. bronchisepticus* infections in animals, and T. Smith (1913–14) has pointed out that *B. bronchisepticus* is a very common infection among animals and that this organism may be found located among the cilia of the ciliated epithelium of the respiratory tract, just as *B. pertussis* is stated to be in whooping cough in man. Hence care is necessary in the interpretation of inoculation experiments with *B. pertussis* in animals.

The portal of entry of *B. pertussis* into the body is presumably by the respiratory tract, and its only location in the body is in that region. Infection is brought about by inhalation of the droplets of sputum containing *B. pertussis* disseminated by the coughing patient.

An attack of whooping cough in childhood confers considerable protection, so that second attacks are the exception in the young and in young adults. But second attacks are not uncommon among the middle-aged and elderly.

It has often been stated that animals, e.g. the cat and dog, may contract whooping cough, but this is probably incorrect. The production of cough in animals by feeding with sputum or by inoculation of cultures has also been reported. Such a result, if it occurs, is probably due merely to the irritant action of the agent and not to infection. Wollstein inoculated cultures into the trachea of monkeys, and sprayed cultures into the nose and throat of dogs and cats with entirely negative results. In one instance, two dogs after the treatment developed snuffles and

sneezing, without cough, but it was afterwards found that they were suffering from distemper—a demonstration of the care necessary in interpreting experiments of this kind.

The evidence incriminating *B. pertussis* as the specific cause of whooping cough is the following: The organism is distinct from all other known species of bacteria. It is uniformly present in whooping cough, is most abundant in the early and most infectious stages of the disease and becomes less and less numerous during the course of the disease, in accordance with the decline of infectivity. The serum of cases sometimes agglutinates the organism, though it is true this phenomenon is not a marked feature. More significant is the constancy with which complement fixation may be obtained with the serum of patients, using *B. pertussis* as antigen, in the course of the disease and for some time afterwards; and the manner in which the complement-fixing substances appear, increase, attain a maximum, and slowly decline in amount is characteristic, and very similar to what occurs in some other infective diseases (see p. 404).

Laboratory Diagnosis of Whooping Cough.

All observers are agreed that it is impossible to recognize with certainty *B. pertussis* microscopically in a stained film of the sputum, owing to the very frequent presence of other and similar organisms, particularly *B. influenzæ*, so that this simple means of diagnosis is not available.

Agglutination of *B. pertussis* by the patient's serum is also a phenomenon too inconstant to be applied as a routine for diagnostic purposes. This at least was the experience of Bordet and Gengou (1909) and of Wollstein (1909). Winholt (1915) found that the agglutinating titre of the serum of cases of 14 days' duration varied between 1 : 10 and 1 : 240, and of 3 to 4 weeks' duration between 1 : 50 and 1 : 150; for a longer duration the titre never exceeded 1 : 70, and was generally much less. Povitzky and Worth (1916) found that of adults' serum, 50 per cent. did not agglutinate, 33 per cent. agglutinated up to 1 : 17, and 17 per cent. up to 1 : 100. Of children not suffering from whooping cough, 40 per cent. agglutinated up to 1 : 40 and 60 per cent. did not agglutinate. In the case of whooping-cough patients, Table II shows the results obtained according to the duration of the disease:

Table II.

Weeks of Whooping	*No. of Cases*	*Not Agglutinating*	*Agglutinating with Dilutions up to :* 1 : 40	1 : 100	1 : 200
1	13	5	4	0	4 *Cases*
2	10	0	6	3	1 ,,
3	9	1	0	6	2 ,,
4	13	2	4	6	1 ,,
6	4	0	4	0	0 ,,

Povitzky and Worth conclude that agglutination cannot be depended upon for purpose of diagnosis in dilution of the patient's serum of less than 1 : 100. Should the reaction with a dilution of 1 : 100 be prompt and complete, the case may be regarded as suspicious, while a complete reaction with a dilution of 1 : 200 probably points to a recent infection with whooping cough. A negative agglutination test has, however, no value whatever.

Complement fixation cannot be employed for diagnosis in the early stages of the disease, but may be of service later on for the determination of cases which are not clinically characteristic. Complement fixation begins to be apparent at the end of the first or second week, then steadily increases in degree until it reaches a maximum in the fifth to the seventh week, after which it slowly declines. Chievitz and Meyer (1916) state that in the second week the serum of some 25 per cent. of patients gives complement fixation, in the third week 70 per cent. of patients are positive, and by the fourth week most patients give the reaction. They found that of 152 cases, 104 gave complement fixation. Winholt (1915) obtained much the same results, and found the reaction to be well marked three months, and still to be present though not so marked eight months, after the attack. The reaction has disappeared within a year or eighteen months after the attack.

Lastly, the method of cultivation may be made use of. This, though presenting some difficulty, has the advantage that it is most successful in the early stages of the disease; it becomes, in fact, less and less successful the longer the disease has existed. The culture method was initiated by Chievitz and Meyer (1916), and the procedure devised by them has since been adopted with little modification. The method has been extensively applied in Denmark.

For the culture method, it is most important to make use of the proper portion of the expectoration. This consists of the thick viscid pellets which are voided at the end of the paroxysm of coughing; the general buccal and nasal mucoid secretion does not usually yield successful cultures. Two methods were adopted by Chievitz and Meyer for obtaining and sowing the expectoration. In the first method, the tongue of the patient is depressed with a spatula. This procedure usually induces a fit of coughing, and at the end of the paroxysm a little of the expectoration is obtained on a sterile cotton-wool swab. This is carefully rinsed two or three times in sterile saline, and the expectoration is then spread over the surface of a plate of the special Bordet-Gengou medium, or some modification of it. This method is by no means an easy one, particularly in the case of infants, and requires the help of a doctor or of some experienced person. The second method is to hold a plate of the special medium in front of the mouth of the coughing patient, at a distance of 10 to 15 cm. The droplets of sputum voided and caught on the plate will usually give after incubation a good growth of the *B. pertussis* if it is

present. By adopting this method, it has been possible in Denmark to institute a system of bacteriological diagnosis, and the following summary of the procedure is taken almost verbatim from Madsen's account of it (1925) : The culture medium employed is a modified Bordet-Gengou one (vide Vol. IX), which is put up in dishes of aluminium, 8 cm. in diameter and 1 cm. deep. The bottom and junction between bottom and side are grooved, so that the medium adheres better. If the medium is properly made, the charged plates may be posted in a suitable wrapping without injury or detachment of the medium. The medium must be used fresh ; it may be kept a fortnight on ice, but only a few days at room temperature.

The sampling is generally done by a relative of the patient. When an attack of coughing occurs, the lid is removed and the dish is held for a quarter of a minute in front of the patient's mouth and about 10 cm. away, so that he coughs (but does not spit) on to the surface of the agar. The dish is then returned as soon as possible to the laboratory. Here it is incubated at 37° C., and after two days, among a varied assortment of all kinds of colonies, the small pearly colonies of the *B. pertussis* will appear if the patient is suffering from whooping cough. In the early stages of the disease the plate may be almost covered with the specific colonies, but later it may be difficult to recognize them among the many colonies of other organisms that grow. With practice, however, the investigator will usually be able to identify the specific colonies even in these circumstances. In addition, confirmation of the naked-eye diagnosis is sought by a microscopical examination of a film prepared from a likely colony, stained by Gram's method and counterstained with dilute carbol-fuchsine, the characteristic organism being looked for. It is important to remember that the *pertussis* colonies do not always have the characteristic appearance described ; the pearly lustre is not always pronounced, and colonies are sometimes found very white in colour and sliding over the surface of the medium when touched with the platinum wire, so that they simulate colonies of *Micrococcus catarrhalis*. These different colony forms are not due to differences in the strain of *B. pertussis* growing, but are caused by some slight variation in the composition of the culture medium, and particularly if it is somewhat dry. Should the macroscopic or microscopic appearance of the colonies on the 'cough' plates be insufficiently typical to ensure a reliable diagnosis, it is useful to sow some of the culture on a new plate, so that the growth may be compared with that of a typical *B. pertussis*. No other bacterial form has so far been found which, in pure culture on potato-blood-agar, resembles the whooping-cough bacillus, and which further investigation has proved to be different from that bacillus.

The result of the examination cannot be obtained earlier than two days from the commencement of incubation of the plate, and three days sometimes elapse, and occasionally four, before the colonies attain sufficient size to determine the species of micro-organism present. This

delay is obviously a disadvantage if practical methods of prevention are to be applied on a basis of the bacteriological diagnosis, but no medium has so far been devised which will hasten the appearance of the characteristic colonies.

Lawson and Mueller (1927), who have made an extensive study of whooping cough at Boston, U.S.A., have adopted much the same methods as those described by Madsen, and the culture medium employed was very similar to the Danish one. They conclude that if the characteristic colonies be found on the plates, a diagnosis of whooping cough may be made with confidence. If, however, the cultures be negative, fresh cultures should be made on three successive days, preferably in the evening, when the cough is most frequent and pronounced. If these three cultures are negative, it is a fair presumption that the condition is not whooping cough, provided that the examination is made during the first three weeks of the disease, and that a satisfactory and freshly prepared culture medium has been employed.

Cutaneous reaction. Some reports have been published, e.g. by Orgel (1922), of the successful use of the cutaneous reaction by the intradermal inoculation of an emulsion of *B. pertussis* for the diagnosis of whooping cough, but neither Riesenfeld (1923) nor Hull and Nauss (1923) obtained successful results by this method.

Immunization and its Practical Applications in Prevention and Treatment of Whooping Cough.

Serum Therapy.

Although some of the earlier results obtained seemed favourable, the general opinion now is that treatment of whooping cough with an anti-serum is of little value. In the favourable reports the amount of serum employed was generally so considerable that its use on a large scale would be impracticable. Klimenko (1912), for instance, prepared a serum by the immunization of various animals, and reported that benefit was derived when doses of 25 to 50 c.cm. were repeatedly administered to the patients.

It has also been claimed that non-immune serum, e.g. ox or human, is of value for treatment ; if so, conceivably by a non-specific protein reaction. The same agent has also been employed for prevention (e.g. Gillot, 1925).

Vaccine Therapy.

Vaccines consisting of dead cultures of *B. pertussis* have been extensively employed both for prevention and for treatment of whooping cough, and these two aspects of vaccine therapy may be considered separately.

The vaccine is prepared by emulsifying the growth of *B. pertussis* obtained on a 48-hours' culture on blood-agar or other suitable medium in saline, with the addition of 0·5 per cent. of phenol as a preservative. The vaccine is standardized to contain a known number of organisms per cubic centimetre, usually 10,000 millions.

Such a vaccine injected into animals causes the production of agglutinins and complement-fixing substances in their serum. In children inoculated with the vaccine, Huenekens (1917) found complement-fixing bodies present in 25 to 50 per cent. Kristensen and Larsen (1926) state that after three doses of vaccine (0·5 c.cm., 0·7 c.cm., and 1·0 c.cm.) given at intervals of four days, complement-fixing substances appear in considerable amount, being at a maximum eight days after the last injection and then diminishing steadily so that they are reduced to half fourteen days later.

Prophylaxis. Conflicting reports of the value of prophylactic vaccination have been published, some regarding it as being of little or no value, others maintaining that it possesses considerable preventive power. No absolutely certain prophylactic effect has been obtained, but the majority of those who have used it agree that cases of infection among the vaccinated are fewer than among the unvaccinated. Thus Hess (1914) reports that of 244 vaccinated children, 20 developed whooping cough; while during the same period, of 80 non-vaccinated children, 59 developed whooping cough, and Luttinger (1915 and 1917) states that of 239 children vaccinated, 216 (90 per cent.) appeared to be protected. Madsen reports the results obtained with prophylactic vaccination in the Faroe Islands, which are subject periodically to severe epidemics of whooping cough. A new and severe epidemic commenced in 1923, and over 2,000 individuals were forthwith vaccinated. In one district 20 per cent. of those vaccinated escaped whooping cough, while of 108 non-vaccinated only two failed to catch the disease.

It was mentioned above that in a vaccinated individual complement-fixing substances begin to diminish in amount ten days after the last inoculation, so that it is not unlikely that any protection afforded by the vaccine soon declines. All recent work also suggests that the vaccine to be of use must be given in considerable doses at shortish intervals. Madsen recommends doses of 0·5 c.cm., 0·7 c.cm., and 1·0 c.cm. at intervals of four days of a vaccine containing 10,000 million organisms per cubic centimetre. When administered subcutaneously, some infiltration at the site of inoculation and slight fever follow; occasionally considerable fever results. Intramuscular inoculation has also been tried and appears to be followed by a less severe reaction. Kristensen and Larsen (1926) have also compared the amount of complement-fixing substances which develop following three intramuscular injections of 0·5, 0·8 and 1·1 c.cm. given on alternate days, and five daily intramuscular injections of 0·1, 0·2, 0·4, 0·7 and 1·0 c.cm. No material difference was observed between the two series, or between them and a series receiving subcutaneous inoculations.

Bloom (1925) as a result of considerable experience of whooping-cough vaccination believes that *pertussis* vaccine confers considerable protection against attack, and that the immunity may be maintained. He gives

1·0 c.cm. of the vaccine on three alternate days, and then 1·0 c.cm. every second year. The vaccine is a mixed one containing 5,000 million *B. pertussis* and 3,500 million *B. influenzæ* per c.cm. In one instance, of 338 vaccinated children only 4 contracted whooping cough, although at least 70 per cent. were exposed to infection. He is emphatic that the vaccine employed should be a freshly prepared one—not more than 10 days old.

Another advantage claimed for prophylactic vaccination, even more important than its possible protective action against infection, is that those who afterwards contract the disease tend to have it in a milder and less fatal form than those who are not vaccinated. Thus, in the Faroe Islands epidemic mentioned above, among 2,094 vaccinated individuals there were 5 deaths, and among 627 non-vaccinated there were 18 deaths. The mortality among the unvaccinated was, therefore, twelve times as great as among the vaccinated, and the opinion was that the attacks were much more severe on an average among the unvaccinated.

Treatment. Vaccine treatment of whooping cough seems to have been initiated by J. Freeman in 1909. Then in 1913, Nicolle and Conor employed a *living* vaccine in the treatment of an outbreak at Tunis. Cultures on potato-blood-agar were emulsified in saline, the organisms were washed several times and finally suspended in saline, the suspension containing 400 million organisms in one drop. The vaccine was injected subcutaneously every 2 to 3 days as often as necessary, the dose being from 1 drop to 5 drops. It was claimed that 78 per cent. of the cases were cured in from 3 to 12 days.

Since then, vaccines containing dead bacteria have been always used for treatment, sometimes consisting of *B. pertussis* alone, sometimes with *B. influenzæ* and other organisms in addition, but it is very difficult to assess the value of the treatment as the subject does not lend itself well to statistical investigation, and the evidence rests mainly on personal opinions based on clinical experience.

Views unfavourable to vaccine treatment have been expressed by Von Sholly, Blum, and Smith (1917), who state that an influenza vaccine or even milk-coloured water gave as good results as a *pertussis* vaccine; by Paterson and Smellie (1922), who employed a mixed *pertussis*, influenza, and pneumococcus vaccine, the dosage, however, being small and not closely spaced; and by Hess (1914).

Reports favourable to vaccine treatment are numerous. Luttinger (1915 and 1917) concludes that vaccine treatment lessens the severity of the paroxysmal stage and shortens its duration, which averaged 25 days in the vaccinated cases compared with 40 days in the unvaccinated group. The best results were obtained when treatment was started in the first or second week of cough with a dose of 250 million organisms, and the dose doubled every other day provided reaction was not excessive, four doses in all being given.

Herrman and Bell (1924) in a careful study of 300 cases of whooping cough, state that the most promising specific treatment is by means of a *pertussis* vaccine. In 25 per cent. of the cases improvement was so marked ' that there was no doubt as to the specific effect '.

Based on a personal experience of thirteen years, Bloom (1925) considers vaccine treatment the most efficient of all forms of treatment. He prefers a freshly prepared vaccine containing 5,000 million *B. pertussis* and 3,500 million *B. influenzæ* per c.cm. Doses of 1·0 c.cm. are given on alternate days until the most prominent symptom abates, and are afterwards continued on every third or fourth day until the other pronounced symptoms diminish. With this method of treatment, the duration of the disease averaged 24 days. Blasi (1927) also reports a much shorter duration as a result of vaccine treatment.

Many clinicians regard the age of the vaccine as being an important factor, a freshly prepared vaccine giving more favourable results than an old one (e.g. R. G. Freeman, 1920, and Moody, 1920), and Huenekens (1927) found that freshly prepared vaccines are more antigenic than stored ones, but Mishulow, Oldenbusch, and Scholl (1927) found that *pertussis* vaccines remain antigenically potent for long periods.

It is evident that, until much more evidence is available, it is impossible to assess properly the value of specific vaccine treatment in whooping cough. Such evidence could probably only be collected by ' team ' work, but in view of the disabilities and mortality caused by whooping cough it would be of great value.

Prevention of Whooping Cough.

Any method of treatment which diminishes the duration of the disease will also be of value in prevention by lessening the period of infectivity, and for this purpose vaccine treatment of the disease at the earliest possible moment is at least worthy of trial.

For prevention proper, the two methods at our disposal are prophylactic vaccination before attack, and isolation of the sick. Prophylactic vaccination would be applicable in an institution or an isolated district, when the disease has been introduced and is likely to spread widely among a susceptible population.

Isolation is a well-established measure, which, with prophylactic vaccination, may be anticipated to limit considerably the incidence of whooping cough. But efficient isolation presents difficulties, one of which is the long duration of the illness. Valuable information has been derived from the bacteriological investigations on whooping cough in Denmark and in the United States on the probable duration of infectivity. In the first place, it must be remembered that the disease commences with an initial catarrhal stage of a week's duration or thereabouts, and that when the ' whoop ' begins, the patient has entered the second week of the disease in an average case. Now Chievitz and Meyer (1916) found that a

large proportion of the cases, say 80 per cent., yielded cultures of *B. pertussis* during the first fortnight of the cough, but that after the cough had lasted four or five weeks it was exceptional to obtain positive cultures. Martin Kristensen (1927) gives the following data, Table III, of the frequency of the presence of *B. pertussis* in 914 patients at different stages of the disease, based upon the results obtained at the Danish diagnostic stations :

TABLE III.

	No. of Cases	*No. in which Bacillus found*	*Percentage Positive*
Catarrhal Stage	134	100	75
Paroxysmal Stage, 1st week	277	158	57
„ „ , 2nd week	201	122	61
„ „ , 3rd week	121	55	45
„ „ , 4th week	74	30	40·5
„ „ , 5th week and later ..	107	10	9·0

These figures show that the bacilli may be found in considerable numbers for quite a long time, and then disappear completely in most cases during the fifth and sixth weeks. In consequence of these results, Madsen states that school children in Denmark are now allowed to return to school after the spasmodic cough has lasted for four weeks, and that this regulation has proved expedient, for the schools have not reported fresh cases of whooping cough caught from the whooping-cough convalescents thus admitted. A limited isolation of this kind would obviously be an enormous gain, for the spasmodic cough may frequently last for many weeks, and parents would, moreover, probably be willing to adopt stricter isolation if they knew that the patients would be released after a month or five weeks' quarantine.

The experience in America is similar. Lawson and Mueller (1927) state that cultures made during the first or catarrhal week usually show large numbers of characteristic colonies of *B. pertussis*, and that during the later stages the number of colonies progressively diminishes. As whooping cough is most infectious during the catarrhal and early paroxysmal stages, Lawson and Mueller contend that a lengthy quarantine is often unjust and irrational, failing to protect the community during the early highly infectious stage, and needlessly restricting the liberty of the patients when they are no longer a source of danger. In theory, isolation for 30 days from the onset of the first catarrhal symptoms would be ideal, and would probably control the spread of infection in over 90 per cent. of the cases. As a further safeguard, ' release cultures ', as in diphtheria, might be made use of, the patient being released from quarantine only when three cultures taken on successive days prove to be negative.

B. pertussis being an organism of feeble vitality apart from the patient, little need be done in the way of disinfection beyond ordinary cleanliness.

It would be wise to disinfect the expectoration and vomit and any articles, handkerchiefs, and clothing and bedding soiled therewith, as the bacillus may live for three days in the dry sputum. In practice, ordinary laundering will probably suffice for clothing and bedding. Chievitz and Meyer (1916) found that a suspension of *B. pertussis* was killed by 2 per cent. phenol only after 30 minutes exposure, but that 1–4,000 mercuric chloride was soon fatal.

Soft Chancre or Chancroid.

It was not until the middle of the last century that the ' soft chancre ' or ' chancroid ' was clearly differentiated from the hard or Hunterian chancre, the primary lesion of syphilis. It is a venereal affection, transmissible by inoculation, but unlike the primary syphilitic sore, which is followed by general systemic infection, it remains a local infection, spreading at furthest to the neighbouring lymphatic glands.

Ducrey in August, 1889, announced that he had found a characteristic micro-organism present in the soft chancre, and he described its appearance and occurrence, the results of the inoculation with chancroid discharge, and his attempts at cultivation, which were unsuccessful, in a series of papers (1890). The organism, a small bacillus, forms pairs and chains, and occurs admixed with many other organisms in the ulcerating chancre, but cannot usually be found in the pus of the secondary bubo. Ducrey succeeded in eliminating the secondary organisms in the following manner: The discharge from the chancre is inoculated on to the forearm, the inoculated area being carefully protected with a sterile watch-glass kept in position by means of a dressing and bandage. After from three to five days a pustule has formed from which a second inoculation may be made in the same way. Ducrey was able in this manner to transmit three separate chancres each through 15 generations. After five or six transmissions, the extraneous organisms have generally disappeared and Ducrey's bacillus will alone be present. Ducrey's findings were soon confirmed by other observers, e.g. Nicolle, Unna (1892), Krefting (1892), and Cheinisse (1894), but it was not until 1900 that the organism was obtained in pure culture by Besançon, Griffon and le Sourd.

Ducrey's Bacillus, *Bacillus ducreyii.*

Morphology.

Ducrey's bacillus (*Bacillus ducreyii, Hemophilus ducreyii*) is a small rod with rounded ends measuring about $1 \cdot 5\mu$ in length by $0 \cdot 5\mu$ in breadth. Its extreme range of variation is from $0 \cdot 5$ to $2 \cdot 5\mu$ long by $0 \cdot 3$ to $1 \cdot 0\mu$ broad, and the organism frequently is constricted at the middle. In the discharge of the ulcerating chancre it occurs singly, in pairs, in small groups, and exceptionally in short chains. In the chancre, at the junction between the living and the dead tissue, it occurs as a strepto-bacillus

forming characteristic chains of four, six, ten or more, individuals. Under cultivation, the organism on a solid medium such as blood-agar retains much the same morphological characters as in the discharge of the chancre, but in the condensation water of such a culture or in a fluid culture medium it assumes the strepto-bacillary form, and long chains may occur.

Teague and Deibert (1924) describe the development of involution forms when the organism is grown on tubes of entire clotted rabbit's blood which has been heated to 64° C. for ten minutes. These take the form of comparatively large, thin and thick rods attached in chains, with some irregularity of shape, and quite different from the typical strepto-bacillary form.

Ducrey's bacillus is Gram-negative, non-motile, and non-sporing. It is best stained with phenol-toluidine blue, and frequently shows polar staining.

Cultivation.

B. ducreyii is ordinarily a strict aerobe, and develops only on a culture medium containing blood, or, less readily, serum. Ducrey tried in vain to cultivate it on all the ordinary culture media, including serum. Besançon, Griffon, and le Sourd (1900) first obtained it in pure culture by the use of an agar with which blood was mixed, also in liquid rabbit serum, and all subsequent investigators have found some form of blood medium to be the most successful. Himmel (1901) used clotted guinea-pig blood, and Stein (1908), Reenstierna (1923), Nicolle and Durand (1924), and Durand (1926) employed various modifications of blood-agar.

B. ducreyii usually dies out within 2 to 5 days unless reinoculated, but Reenstierna found that it will maintain its vitality for 6 weeks on a *soft* nutrient agar (agar, 2·5 per 1,000) containing one-fifth of its volume of defibrinated rabbit's blood.

Nicolau and Banciu (1926) find that while the presence of blood is at first an indispensable condition for the cultivation of *B. ducreyii*, the red corpuscles being the essential element, at the end of a variable period of gradual acclimatization it will develop on ordinary laboratory media without blood or even serum, though a small addition of the latter is to be preferred, the optimum pH being 7·2. When this state is reached it will also develop anaerobically on a soft agar (agar, 3 per 1,000) containing some horse-serum.

Teague and Deibert (1924) tested various mixtures of aqueous agar, red-cell extract, and blood-serum, for their capacity to support the growth of Ducrey's bacillus. The agar consisted of 2 per cent. of agar with 0·5 per cent. of sodium chloride in distilled water. The red-cell extract consisted of 2 c.cm. of washed red-blood corpuscles added to 10 c.cm. of boiling physiological salt solution, and the mixture boiled for three minutes; the supernatant fluid constituted the red-cell extract. The

blood-serum was fresh rabbit's or sheep's serum heated to 55° C. for 15 minutes. The following results were obtained :

Agar + rabbit serum = no growth.
Agar + rabbit serum + peptone = good growth.
Agar + rabbit serum + peptone + red-cell extract = excellent growth.
Agar + red-cell extract = no growth.
Agar + sheep serum = no growth.
Agar + sheep serum + peptone = no growth.
Agar + sheep serum + peptone + red-cell extract = excellent growth.

The colonies of *B. ducreyii* are surrounded by a faint zone of hæmolysis after 48 hours' incubation, which becomes well marked after three or four days.

Saelhof (1924) describes the appearance of the colonies on a blood-agar medium as follows : In 24 hours, they appear as small, round, slightly elevated, mucoid-looking dots, pin-point in size, and without visible change in the medium. After 48 hours, they are 0·25 mm. in diameter, round, slightly elevated and translucent, and surrounded with a slight zone of hæmolysis. Later, the hæmolysis becomes well marked, and the colonies become whitish or milky, and acquire a refractile metallic sheen.

B. ducreyii does not liquefy blood-clot ; its other biochemical activities do not appear to have been investigated. [De Assis (1927) states that bacilli apparently identical with *B. ducreyii* isolated from the normal urethra produced acid in glucose, lævulose, galactose, lactose, maltose and saccharose. Two strains also produced acid from mannitol, but none fermented dulcitol, inulin and glycerol.] Nicolau and Banciu state that the acclimatized organism acidifies and coagulates milk.

The method of isolation is given under ' Diagnosis ', p. 414.

Immunological Reactions.

An antiserum can be prepared by the inoculation of animals with cultures of the *B. ducreyii ;* this will be described later.

Teague and Deibert and also Saelhof attempted to prepare agglutinating sera by the inoculation of rabbits with pure cultures. The results were not very satisfactory ; they tended to be inconstant, and there is difficulty in preparing emulsions of the organism satisfactory for use as antigen. No evidence was obtained of the existence of serological races.

The serum of patients does not appear to agglutinate the organism.

Pathogenic Action.

B. ducreyii possesses little pathogenic action except for man, and for him only to the limited extent seen in the disease.

The chancroid is frequently multiple, and is auto-inoculable as already mentioned ; there is no immunity as a result of infection. Ricord and others showed that after inoculation has been repeated a certain number

of times, the individual may become insusceptible. Some individuals never acquire an immunity, and in all it is only temporary. Infection of the neighbouring lymphatic glands with the formation of buboes, which frequently suppurate, is a common occurrence. The severe spreading, destructive and phagedænic ulcers which sometimes supervene are the result of secondary infections.

Chancroid appears after a variable but short incubation period, which in auto-inoculation lesions is about 24 hours. Pus of a chancroid applied to the skin is not followed by infection unless there is a breach of continuity in the skin. If the pus be injected into the subcutaneous tissue, an abscess results. Both the pus of the human lesions and pure cultures of *B. ducreyii* are almost without action upon animals. Saelhof (1924) obtained entirely negative results in a number of rabbits inoculated with pure cultures cutaneously, subcutaneously, and into the inguinal glands. With monkeys, in a small number of instances chancroid was produced by inoculation with pure culture.

Carriers of *B. ducreyii* exist, for Saelhof and also Brams (1924) isolated it from the smegma of non-chancroid individuals. Brams investigated 30 phimotic cases with large collections of foul-smelling smegma, and isolated the organism from five of the cases. De Assis (1927) also states that he has isolated from the normal male urethra streptobacilli apparently identical with Ducrey's bacillus.

Laboratory Diagnosis of Chancroid.

Teague and Deibert (1920) have investigated the application of laboratory methods for the diagnosis of chancroid. Ducrey's bacillus appears in the tissue of the chancroid in characteristic chains of small Gram-negative bacilli, and as similar chains of non-pathogenic organisms are not found (or are very rare) in non-venereal ulcers, it would seem a simple matter to make a diagnosis by the examination of stained smears. But, as already mentioned, Ducrey's bacillus passing from the tissues into the purulent discharge of the ulcer, loses its characters, appearing for the most part as bacilli, single or in pairs, and rarely in chains, even short ones. It is, therefore, unsafe to make a diagnosis from the examination of a stained smear unless characteristic chains be found, though the presence of small Gram-negative bacilli, singly, in pairs, or in small groups, may furnish considerable evidence in favour of the lesion being chancroid.

The method of diagnosis finally adopted by Teague and Deibert was a culture one. The culture medium employed is a comparatively simple one and is prepared as follows : Blood is withdrawn aseptically from the heart of a rabbit with a 20 c.cm. syringe, and is immediately distributed into small sterile test-tubes, measuring 10 cm. long by 10 mm. in diameter, slightly less than 1·0 c.cm. of blood in each. The tubes are allowed to remain at room temperature until the blood has clotted, and are then heated to 55° C. for five minutes. They should be used at once, or if kept

on ice may be used the following day. If not heated but kept on ice, the tubes may be used up to 3 to 5 days after preparation. Rabbit blood is preferable to human blood. The inoculum is obtained by means of pieces of stiff iron wire (gauge 18) $5\frac{1}{2}$ inches long, the terminal $\frac{1}{8}$ inch being bent on itself, which are sterilized. The bent end of the wire is rubbed gently over the base of the ulcer and under its undermined edge, and a bead of the discharge is picked up. This is inoculated into a clotted-blood tube, the wire being passed several times round the clot so as to distribute the discharge in the serum. A second tube is similarly inoculated with a freshly charged wire. The tubes are incubated at 37° C. for 24 to 48 hours and are then examined. This is done by thoroughly stirring the serum around the clot with a platinum loop and then making smears. These are stained by Gram's method and are examined microscopically. Ducrey's bacillus appears in the form of characteristic chains and tangles of small Gram-negative bacilli, sometimes pure, sometimes admixed with Gram-positive bacilli and cocci. If these characteristic chains and tangles of small Gram-negative bacilli be found, the culture may be regarded with confidence as being positive for Ducrey's bacillus. It was found better not to wash the ulcer with saline before taking the material for inoculation.

The results obtained by this method were as follows : Ducrey's bacillus was found in 140 cases out of 274 genital sores examined. Of the 134 negatives, 69 were further investigated and followed up. Of these, 63 were almost certainly not chancroid, being syphilitic, herpetic, &c., leaving 6 probably chancroid, but not diagnosed as such. This result indicates that 90 per cent. of cases can be diagnosed by this culture method. Recovery of the organism from the buboes is difficult and uncertain.

Cutireaction. Ito in 1913 showed that a cutaneous reaction is obtainable in chancroid. Reenstierna (1923) has developed the method and finds it to be characteristic and reliable. The material employed is a culture of Ducrey's bacillus on a blood-agar slope grown for 24 hours at 37° C. The growth is emulsified in saline containing 0·5 per cent. of phenol, one loopful of the growth in 1 c.cm. The emulsion is kept on ice for 14 days, and may then be used, 1 c.cm. being injected into the cutis with a fine needle, a control being done at the same time with phenolized saline.

The specific reaction should be apparent 48 hours after injection ; occasionally a non-specific reaction may develop on the day following inoculation, but will have disappeared by the next day. Of 142 cases of bubo with or without ulceration, all except 8 were strongly positive ; of 31 cases with ulcers only, all except 3 were positive. Individuals who have had the disease may react months or years afterwards. Nearly all of 229 controls without history of chancre were clearly negative, a few being doubtful.

The Nicolle-Durand vaccine (see below, p. 416) may also be employed for the cutaneous reaction.

Specific Treatment of Chancroid with Vaccine and Antiserum.

Cruveilhier (1922) found that if chancroid pus be suspended in saline and heated to 57° C. for half an hour, it no longer infects on injection but acts as a vaccine and tends to cure the chancroid.

Nicolle and Durand (1924) claim brilliant curative results by the use of a vaccine prepared as follows: Ducrey's bacillus is grown on a blood-agar slope for 24 to 36 hours. The growth is then emulsified in saline and the emulsion is centrifuged so as to deposit the organisms. The supernatant fluid is pipetted off and discarded, and the deposit is again suspended in saline, glass beads are added and the mixture is well shaken, and this process of centrifuging, &c., and shaking with glass beads, is repeated again. The final deposit obtained after centrifuging the second shaken mixture is finally emulsified in saline containing 2 per cent. of phenol, and is standardized to an opacity corresponding to 450 million typhoid bacilli per c.cm., as it is impossible directly to count the number of organisms in the emulsion. For use, this emulsion is diluted with an equal volume of phenolized saline and is administered by intravenous inoculation. The system of dosage to be preferred is to give 0·75 c.cm., 1·0 c.cm., 1·5 c.cm., and 2·0 c.cm. at intervals of 2 to 4 days; rebellious cases may receive an additional dose of 3·0 c.cm. This treatment was found to be innocuous and to cause no reaction, and was most efficacious, simple chancres and buboes, even when inflamed and fluctuating, resolving in 12 days or so on an average. Infected and phagedænic conditions and open buboes were less surely affected. Mazza confirms the value of the foregoing method of Nicolle and Durand, and states that an autovaccine gives brilliant results.

De Assis and Fraga (1926) state that they have successfully treated chancroid with compresses soaked in the filtrate of a culture of Ducrey's bacillus in a glycerinated egg-broth of Besredka grown for 18 days at 37° C.

Reenstierna has employed an antiserum for treatment. The serum is prepared by inoculating rams intravenously with saline emulsions of blood-agar cultures of Ducrey's bacillus, sometimes living, sometimes dead. It is necessary to commence with a very small dose and to increase it slowly and gradually, injecting every 2 to 3 days. The treatment of the animal occupies 6 weeks, and it is bled 9 days after the last injection. This serum gave fixation of complement with an amount as low as 0·005 c.cm. For treatment, two or sometimes three intramuscular injections of 10 c.cm. were given at intervals of 3 to 5 days. Most unopened and non-incised buboes heal rapidly under the treatment—within 5 to 10 days. The serum also exercises a very favourable influence upon the chancres.

The Morax-Axenfeld Bacillus.

The Morax-Axenfeld bacillus, the cause of a subacute form of conjunctivitis ('diplo-bacillary conjunctivitis'), was first described by Morax in 1896, and Axenfeld stated in a paper published the next year

(1897) that he had independently observed the same organism some months previous to his publication. If priority of publication be the criterion, the credit for this discovery belongs to Morax alone.

The Morax-Axenfeld bacillus (*Hemophilus lacunatus, Bacillus lacunatus, Bacillus duplex*) is a short, stumpy bacillus, measuring 2 to 3μ long, by 1 to 1·5μ broad. In the discharge, it occurs for the most part in pairs, end to end, but is occasionally single, and occasionally it forms short chains consisting of six to eight elements. It lies free between the pus cells, or some of the bacilli may be intracellular. The organism is non-motile, non-sporing, is not encapsuled, and is Gram-negative. It is an obligate aerobe, and does not grow on ordinary agar and gelatin, nor in broth.

Morax succeeded in cultivating and isolating the bacillus on an ascitic-fluid agar, and found that it grew also in ascitic-fluid broth. The organism is easily isolated, and grows well, on a ' digest ' type of medium, e.g. Fildes' peptic blood-agar as used for *B. influenzæ*. In 24 hours the colonies of the bacillus form little grey, hardly visible spots, resembling those of a streptococcus, or more opaque like those of a diphtheroid. The colonies increase in size with further incubation, and after 5 to 6 days may be 2 to 3 mm. in diameter, projections develop on the margin, and eventually they become rosette-shaped. The bacillus grows best at 30 to 37° C., but does not develop below 23° C. It was killed in 15 minutes at 58° C.

Axenfeld likewise found that the bacillus grows on serum- or blood-agar, and in serum-broth, also on agar with an addition of hydrocele or ovarian cyst fluid. He also found that it would grow on inspissated blood-serum. On this medium, after 24 hours' growth, a slight unevenness in the form of small, damp spots, appears, but in two days and later, the surface shows small, round, transparent depressions with slow liquefaction ; from this phenomenon the specific name ' *lacunatus* ' is derived.

Eyre (1900) found that the bacillus would grow on an ascitic-fluid gelatin with slow liquefaction. He also observed that after acclimatization it would grow on ordinary nutrient agar, and that it then gave slight acidity when grown in litmus serum-broth and in litmus milk.

In culture, the Morax-Axenfeld bacillus shows marked pleomorphism ; it may be short and stunted and almost diplococcoid, or take the form of jointed or unjointed threads. On inspissated serum, involution forms become a marked feature after two or three days' incubation—large spherical cells, overgrown diplococci, and dumb-bells prevailing, together with fine pointed spindles, and long, straight, thick or curved rods and filaments.

Morax found that the bacillus produced no effect upon the mouse, guinea-pig, rabbit and pigeon by intraperitoneal, intravenous, and subcutaneous inoculation. Inoculated on the conjunctiva, no effect was produced in the monkey, dog, rabbit and guinea-pig, but in man a typical conjunctivitis followed. Axenfeld and Eyre similarly obtained no pathological effect when the organism was inoculated in animals.

Petit (1900) has described an organism, under the name *Diplobacillus liquefaciens*, very similar to the Morax-Axenfeld bacillus in morphology, but developing on ordinary agar and gelatin, and giving considerable liquefaction in inspissated serum and in gelatin. On ascitic-fluid agar, its colonies are regularly rounded and elevated, while those of the Morax-Axenfeld bacillus have a central boss, i.e. are mammillated. The colonies on ascitic-fluid agar are grey, thick, and viscous. On potato, a yellowish-white, creamy and viscous growth develops. The organism is non-pathogenic to animals, and is met with in certain forms of conjunctivitis, in the secretion of which it is abundant and mostly arranged in pairs like the Morax-Axenfeld bacillus. It measures about 2μ in length by 1·0 to 1·5μ broad, and is Gram-negative, non-motile, and a strict aerobe, and lives for several weeks in culture. It is killed in 15 minutes at 55° C. Taylor (1915) also isolated this organism from the throat.

Another and somewhat similar organism was isolated by Oliver and Wherry (1921). It resembled the Morax-Axenfeld bacillus in most respects but did not liquefy blood-serum; on this account they named it '*Bacillus duplex non-liquefaciens*'. The organism was found in ulcers of the cornea and not infrequently in the sputum of acute and chronic bronchitis.

Zur Nedden (1902 and 1904) isolated from severe corneal ulcers with hypopyon a small Gram-negative aerobic bacillus. It measured about 1·0μ long by 0·6μ broad, showed polar staining, was non-motile and non-sporing. It grew readily on ordinary culture media, giving pearly-white growths on agar and gelatin, without liquefaction of the latter, and a thick, yellowish-brown growth on potato. Milk was slowly curdled, indole was not formed, and dextrose was not fermented. The organism is known as the ' Zur Nedden Bacillus '.

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